Vol. III THE JOURNAL OF NUTRITION No. 3

NOVEMBER, 1930

# IRON REQUIREMENT IN EARLY CHILDHOOD

*By*

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Received for Publication—February 20, 1930

STANDARDS for the iron requirement of the adult man have been established by Sherman (1) and a number of studies of iron balance have been made upon infants, but a careful survey of the literature has revealed no such experiments upon any young children over a year old. The difficulties in such a study are very great. Not only must the most scrupulous care be taken to prevent contamination of samples with iron, but also the management of the young child demands the constant attention of one person throughout the whole experimental period, both day and night, to insure collection of excreta without loss. In addition, the preparation of the diet, the supervision of every meal, that all food be eaten and none spilled or otherwise lost, and the maintenance of the normal daily routine require the full time assistance of at least one other person. It seems worth while, therefore, to report a study of iron metabolism on a child two years and seven months of age, made in April, 1929, under most favorable conditions.

The subject was a healthy girl, born August 26, 1926, who had been for more than two years a member of a group of four children of about the same age, brought up together under expert supervision, primarily for the sake of psychological investigations. At the time of this study she weighed 13.95 kilos. No changes were made in the mode of living, except the necessary presence of one of the investigators at all times during the metabolism experiment. The children's regular supervisor and her assistants were in sympathy with this study and cooperated most cordially, even though inconvenienced by the strict surveillance which it was necessary to maintain over the subject.*

* For this opportunity special thanks are due to Dr. Edith M. Burdick, Psychologist of the

The group lived in a city house, but there was plenty of sunshine in the afternoon, and the children were taken into the back yard or to a near-by park to play whenever the weather permitted. The regular schedule of meals, rest, play, and sleep afforded an ideal situation for a nutrition study.

The experiment lasted twelve days, during which two of us took turns, week about, living in the house and keeping constant vigil over the subject day and night. In order to forestall possible loss of urine, the child was roused several times during her afternoon nap and twice during the night, but readily went to sleep again and showed no ill effects.

It was our purpose to get an iron balance on a normal diet, as near the minimum intake for equilibrium as possible. The child's ordinary diet was an excellent one, well adapted to her age, and the only modification was in the direction of simplification and a slightly reduced iron content. Throughout the experiment she was an ideal subject, eating the unvarying diet without complaint, consuming all of it without compulsion and without mishaps of any kind.

The details of the diet are given in Table I.

All the foods were prepared and weighed by one of us, special precautions being taken against contamination from dust and iron. The foods were exposed only when being transferred to dishes and when being eaten, otherwise being carefully covered by glass covers or celluloid paper.* All utensils were iron-free, of glass, silver or enamel. Distilled water was used in preparing foods and for drinking. Dry toasted "whole wheat" cereal was weighed out for the time and kept in a covered glass jar, from which each day's portion was weighed out and freshly cooked over hot water in a pyrex glass dish. Enough potatoes for several days were steamed in the skins, peeled, mashed with a wooden spoon and put into covered glass jars. The daily portion was warmed with milk and salt, when needed. Canned pea pulp and prune pulp, specially prepared for children, were bought in four ounce glass jars, which were opened as needed. Commercial dried orange juice was diluted with water to about the strength of fresh juice. Fresh certified milk was purchased daily. Bread was procured from a local store and toasted on a chromium toaster as needed. Aliquot samples of all the foods were reserved for analysis in covered glass containers and kept in a refrigerator at 35° F. Urine and feces were collected

Harmon Foundation for Child Study, who kindly permitted us to use one of her group for this study.

* Cellophane.

TABLE I

| Time | Food | Weight (grams) |
|---|---|---|
| 7:00 A.M. | Milk | 230 |
| | Toasted "Whole Wheat" Cereal* (dry) | 20 |
| 8:00 A.M. | Orange powder | 20 |
| | Toast | 10 |
| 11:00 A.M. | Milk | 230 |
| | Pea pulp | 50 |
| | Potato (cooked) | 60 |
| | Prune pulp | 50 |
| 2:00 P.M. | Milk | 230 |
| 5:30 P.M. | Milk | 230 |
| | Toasted "Whole Wheat" Cereal (dry) | 10 |
| | Prune pulp | 50 |
| | Bread, white | 25 |

10 cc. of milk of magnesia were added to 3 pints of milk (1440 gms.), the daily portion plus sample for analysis.

daily in heavy new enamel bowls, free from cracks. The urine was transferred to glass jars, but the feces were kept in the bowls and protected from dust with celluloid paper.

Since the experimental diet differed but little from the ordinary one, the child was kept on the new diet but one day before quantitative collections of excreta were begun. There followed nine perfect days in which no portion of food or excreta was lost. These were divided into three periods of three days each. On the eleventh day, a small portion of urine was lost and it was decided to exclude this day and conclude the experiment instead of running three four-day periods as originally planned.

Iron analyses were made as follows:

1. The entire collection of urine and aliquot samples of milk, peas and prunes, for each three-day period.
2. Two samples each of potato and bread, one representing the first three-day period and the other the second and third periods.
3. One sample each of toasted "whole wheat" cereal and orange powder representing the entire time.
4. Daily collection of feces.

* Wheatena.

Analyses of all foods were made in triplicate. Due to the small amount of iron in the urine, the collection for each three-day period was combined for analysis. Each day's collection of feces was ashed by itself, dissolved and made up to volume, then divided for duplicate or, if possible, triplicate analyses. As there was some difficulty in ashing the feces, the ash was extracted with 1:1 glacial acetic acid, filtered, the filtrate evaporated and the filter paper added and ashed. A small amount of carbon still persisting, from 5 to 8 cc. of concentrated hydrochloric acid and 20 cc. of water were added with heat and the dissolved ash was then filtered into a 100 cc. flask. A check determination was made upon the filter paper, and a correction made for the iron which it was found to contain.

TABLE II

AVERAGE DAILY INTAKE OF IRON

| Food | Weight grams | Iron Intake by Periods | | |
|---|---|---|---|---|
| | | I | II | III |
| | | milligrams | milligrams | milligrams |
| Toasted "Whole Wheat" cereal (dry) | 30 | 0.63 | 0.63 | 0.63 |
| Milk | 920 | 0.55 | 0.74 | 0.83 |
| Orange powder | 20 | 0.15 | 0.15 | 0.15 |
| Potato, cooked | 60 | 0.35 | 0.34 | 0.34 |
| Pea pulp | 50 | 1.59 | 1.48 | 1.45 |
| Prune pulp | 100 | 1.05 | 1.09 | 1.15 |
| Bread, white | 35 | 0.32 | 0.15 | 0.15 |
| Total | | 4.64 | 4.58 | 4.70 |

The laboratory in which all analyses were made was a small room within a larger one, well protected from air currents and dust. The walls were washed free of dust and special precautions were taken that no piece of apparatus used was made of iron. Milk and urine were evaporated on a copper water bath. The foods were ashed in an alberene hood, first over a low free flame and then in a muffle at low red heat. The Zimmermann-Reinhardt method was used for the determination of iron (2).

Since the foods contained but little iron, a small amount of hydrochloric acid was sufficient to dissolve the ash and a correspondingly small final volume for titration was adopted. Analyses of samples, to which were added known amounts of iron, checked within 1 or 2 per cent when this

modification was employed. To the ash was added from 3 to 5 cc. of hydrochloric acid and from 20 to 30 cc. of water. After boiling a few minutes, 1 molar stannous chloride was added drop by drop until the yellow color disappeared, and then 2 drops in excess. The solution was cooled to about 20° C, and 10 cc. of 0.25 molar mercuric chloride added, stirring vigorously. After diluting to from 100 to 125 cc., 10 cc. of "preventive solution"* were added and titrated with potassium permanganate solution of the strength of about 0.4 milligram of iron per cubic centimeter. The intake and output and balance of iron are given in Tables II, III and IV respectively.

TABLE III

AVERAGE DAILY OUTPUT OF IRON

| | Iron Output by Periods | | |
|---|---|---|---|
| | I | II | III |
| | milligrams | milligrams | milligrams |
| Feces | 6.04 | 4.68 | 5.91 |
| Urine | 0.16 | 0.20 | 0.22 |
| Total | 6.20 | 4.88 | 6.13 |

TABLE IV

IRON BALANCE BY PERIODS

| | I | II | III |
|---|---|---|---|
| | milligrams | milligrams | milligrams |
| Iron Intake | 4.64 | 4.58 | 4.70 |
| Iron Output | 6.20 | 4.88 | 6.13 |
| Balance | −1.56 | −0.30 | −1.43 |

The average daily intake was 4.64 milligrams and the average daily balance of iron for the 9 days was negative to the extent of 1.10 milligrams. It will be noted that the fluctuation from period to period was slight, although the intake during the experiment was reduced below the regular diet by the omission of the yolk of one egg and a small portion (½ tablespoon) of bran daily. This leads us to believe that the child was very close to her minimum requirement, which would appear to be about 5.70 milligams.

* Preventive solution has the following composition 2M $H_2SO_4$; 2M $H_3PO_4$; 0.3M $MnSO_4$.

The energy value of the diet, calculated from average analyses, was 1118 calories, or 80 calories per kilo. of body weight and the minimum requirement would therefore be equivalent to about 0.5 milligram per 100 calories. A dietary standard for a child should provide some surplus for growth. If a margin of 50 per cent were allowed, the dietary standard for this child would be 8.50 milligrams, equivalent to 0.76 milligram per 100 calories. It would seem that the allowance of 0.50 milligram per 100 calories would not allow enough margin for a growing child between 2 and 3 years of age.

In a dietary study of pre-school children made by McKay (3), in which the food eaten by each child for a four-day period was weighed by a trained investigator, the estimated iron intake for seven boys in private homes, 2 to 3 years old with an average weight of 13.3 kilos., ranged from 5.33 milligrams to 8.59 milligrams daily; and that for five girls in private homes with an average weight of 14.0 kilos., ranged from 5.53 milligrams to 8.79 milligrams, averaging 6.99 milligrams.

The average energy consumption for the five girls was 1152 calories per day, or 81.8 calories per kilo. of body weight, approximately the same as that of our subject; and their iron intake was 6.99 milligrams daily or 0.6 milligram per 100 calories, which would appear to be very close to minimum requirement.

The average energy consumption for the seven boys was 1249 calories per day or 95.1 calories per kilo. of body weight, their iron intake averaging 7.0 milligrams per day or 0.56 milligram per 100 calories. The iron intake per kilo. of body weight was 0.51 milligram for the boys and 0.49 milligram for the girls. Estimating the requirement of our subject as 5.70 milligrams per day, or 0.40 milligram per kilo. of body weight, it would appear that these other children from good homes were not receiving an amount of iron which afforded a fifty per cent margin of safety, though it may have covered actual requirement. The iron content of our subject's diet, as calculated from Sherman's tables (4),was 6.11 milligrams per day or over 30 per cent higher than the actual amount as determined by analysis. It may also be noted in passing, that a group of children from an orphanage, also studied by McKay, had a daily iron intake which was but little over one-half that of these children from private homes.

In view of recent observations regarding the value of copper in the prevention and cure of nutritional anemia in chicks (5) and young white rats (6), it seemed of interest to estimate the copper intake of our subject.

According to data furnished by Lindow, Elvehjem and Peterson (7), the total copper intake per day was calculated as 0.85 milligram.

In case of rats on a milk diet, it has been shown by Elvehjem, Steenbock and Hart (8) that the addition of 0.005 milligram of copper is sufficient to cure anemia when accompanied by 0.5 milligram of additional iron. Assuming a milk consumption of 35 cc. per rat per day, there would be an intake of about 1 part of copper to 60 of iron. In our subject's diet there would appear to be about 1/5 as much copper as iron. It is hardly thinkable, therefore, that the situation was complicated by any deficiency of copper.

For three days following the last day of collection of samples, hemoglobin determinations were made on the blood by the Newcomer method (9), in duplicate by two observers. The average of all 6 determinations for the three days was 13.7 grams per 100 cc. of blood and the range 13.5 to 14.0 grams. This corresponds to a hemoglobin percentage of about 85, while according to Grigorowa (9), the mean normal hemoglobin percentage in the blood of children from three to four years of age is 65 per cent.

It seems safe to conclude that our subject was receiving daily an amount of iron which was not adequate for all her needs, although up to the standard of 0.5 milligram per 100 calories, and although she was maintaining a level of hemoglobin above the average for supposedly normal children of her age. She was in a state of negative iron balance, and should, apparently, have had an intake about 50 per cent higher, or approximately 0.8 milligram per 100 calories, to maintain equilibrium and provide for growth. It is recommended that children from two to three years of age receive at least 0.75 milligram of iron per 100 calories.

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# THE BASAL METABOLISM OF THE FILIPINOS

*By*

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Received for Publication—February 30, 1930

WHILE environmental factors and diet greatly influence basal metabolism, recent studies by the Carnegie Institution of Washington (Steggerda and Benedict, 1928; Williams and Benedict, 1928), tend to indicate that race may be also an important specific factor to be reckoned with. The present studies, undertaken primarily to set up basal metabolism standards for medical practitioners in the Philippine Islands, are herewith presented as additional contribution to the problem of racial metabolism.

*Subjects and Climatic Conditions.* Our observations were made on 104 healthy subjects, mostly medical students, some laboratory helpers, a few professors and outsiders. The majority was between 20 and 30 years old. Owing to the high degree of intelligence of the subjects, easy cooperation could be obtained without causing undue mental embarrassment.

The Philippine climate is distinctly tropical. It may be divided into three seasons of about four months' duration each, the hot, the rainy, and the cool season. There is plenty of humidity and during the hot season the average temperature of 30° to 35° C. is uncomfortable even for natives of the country. The minimum temperature during the cool season seldom goes down lower than 18° C. early in the morning.

*Methods.* The determinations were made during the 4 months of the cool season. All measurements were made with a carefully calibrated Benedict-Roth respiration metabolimeter. The tests were performed between 8 and 9 o'clock in the morning after one hour of complete muscular and psychic repose in a comfortable bed, the subject having fulfilled the usual prerequisites for such tests, *viz.*, that he shall have taken his last meal 12 hours previously; shall have had a quiet evening of rest; and must be free from fever and mental worries. Determinations were made on each subject on two and sometimes three different days, duplicate tests being made each day. The lower value for any one day was taken as the basal metabolism for that day. The average of these lower values for different days was taken as the metabolism level of the individual. In a few in-

stances the subjects were available for observation only one day. All observations were of 10 minutes duration.

Two subjects are of some particular interest, Nos. 79 and 86 in Table I. These had had their metabolism taken in Winter in the United States after having resided there about one year. Observations subsequently were made a year after their return to the Philippines.

TABLE I

TYPICAL EXAMPLES OF BASAL METABOLISM OF FILIPINO MEN

| Subject No. | Age | Observations | | Body wt. kg. | Height cm. | Pulse rate | $O_2$ used per min. | Heat produced | | | Per cent Deviation from standard | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 24 hours | | per hr. per sq. m. | | |
| | | Days | Periods | | | | | Total Cal. | per kg. | | Harris-Benedict | Aub-DuBois |
| 1 | 14 | 1 | 2 | 44.7 | 161 | 60 | 198 | 1376 | 30.8 | 40.0 | − 1.5 | −13.0 |
| 4 | 16 | 3 | 6 | 55.4 | 162 | 58 | 224 | 1556 | 28.1 | 41.0 | + 1.5 | − 4.6 |
| 8 | 18 | 2 | 4 | 48.5 | 158 | 62 | 203 | 1410 | 29.1 | 40.0 | + 0.5 | − 2.4 |
| 12 | 19 | 2 | 4 | 56.8 | 168 | 62 | 204 | 1417 | 24.9 | 36.2 | − 9.2 | −11.7 |
| 17 | 20 | 2 | 4 | 59.4 | 161 | 48 | 197 | 1369 | 23.0 | 35.1 | −11.9 | −11.1 |
| 22 | 21 | 2 | 4 | 54.4 | 172 | 66 | 217 | 1508 | 27.7 | 38.3 | − 1.7 | − 3.0 |
| 27 | 21 | 2 | 4 | 50.0 | 159 | 63 | 201 | 1397 | 23.7 | 39.0 | − 0.7 | − 1.2 |
| 39 | 22 | 2 | 4 | 56.8 | 164 | 63 | 217 | 1508 | 26.5 | 39.2 | − 0.7 | − 0.7 |
| 41 | 22 | 2 | 4 | 54.7 | 167 | 58 | 205 | 1424 | 26.0 | 37.3 | − 5.4 | − 5.5 |
| 52 | 23 | 2 | 4 | 52.8 | 170 | 56 | 215 | 1494 | 28.3 | 38.9 | + 0.4 | − 1.5 |
| 56 | 23 | 2 | 4 | 54.4 | 163 | 60 | 191 | 1327 | 24.4 | 35.2 | −10.0 | −10.8 |
| 61 | 24 | 2 | 4 | 65.6 | 164 | 75 | 205 | 1424 | 21.7 | 34.9 | −12.4 | −11.6 |
| 72 | 26 | 2 | 4 | 78.0 | 162 | 64 | 246 | 1709 | 21.9 | 38.9 | − 3.6 | − 1.5 |
| 78 | 29 | 3 | 6 | 78.0 | 161 | 64 | 221 | 1536 | 19.7 | 35.1 | −12.1 | −11.1 |
| 79 | 32 | 2 | 4 | 50.0 | 165 | 54 | 161 | 1119 | 22.4 | 30.2 | −17.9 | −23.5 |
| 83 | 34 | 1 | 2 | 65.0 | 171 | 62 | 201 | 1397 | 21.5 | 33.0 | −11.9 | −16.4 |
| 85 | 38 | 2 | 4 | 46.5 | 158 | 74 | 191 | 1327 | 28.5 | 38.4 | + 7.0 | − 2.7 |
| 86 | 41 | 2 | 4 | 75.8 | 169 | 61 | 199 | 1383 | 18.2 | 30.9 | −17.5 | −24.9 |
| 88 | 48 | 1 | 2 | 44.6 | 156 | 66 | 178 | 1237 | 27.7 | 36.8 | + 8.8 | − 4.4 |
| Average of 88 subjects<br>14 were 14 to 19 yrs.<br>55 were 20 to 24 yrs.<br>19 were 25 to 48 yrs. | | | | 53.0 | 163 | 62 | 197 | 1376 | 26.1 | 36.8 | − 5.6 | − 7.8 |

Before beginning a metabolic rate measurement, the apparatus was carefully tested for leaks in a very simple manner which proved so effective that we take liberty in describing it here.

An ordinary spirometer half filled with air is put into communication with the mouthpiece of the empty metabolism apparatus by rubber tubing. The spirometer bell is now depressed to its lowest position of rest, thereby driving the contained air into the metabolimeter, and causing the bell of the latter to rise. An initial reading is taken on the metabolimeter. The bell of the

ordinary spirometer is next raised to a convenient height, and subsequently moved down and up for 3 to 5 minutes causing the metabolimeter bell to move up and down, as in an actual metabolism determination. Then the spirometer bell is brought down once more to the lowest position of rest and a final reading is taken on the metabolimeter. Absence of leak is shown by complete agreement between the initial and final readings. Leaks of almost insignificant magnitude have been detected by this procedure much more readily than by the usual method of placing a weight on top of the raised metabolimeter bell. The latter method requires a longer time to carry out and will not detect a leak of the "inlet valve type."

TABLE II

BASAL METABOLISM OF FILIPINO WOMEN

| Subject No. | Age | Observations | | Body wt. kg. | Height cm. | Pulse rate | $O_2$ used per min. | Heat produced | | | Per cent Deviation from standard | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 23 hours | | per hr. | | |
| | | Days | Periods | | | | | Total Cal. | Per kg. | per sq. m. | Harris-Benedict | Aub-DuBois |
| 1 | 19 | 1 | 2 | 52.0 | 158 | 78 | 197 | 1369 | 26.3 | 37.7 | + 0.9 | − 0.7 |
| 2 | 19 | 2 | 4 | 49.7 | 165 | 67 | 174 | 1209 | 24.3 | 33.1 | −10.2 | −12.9 |
| 3 | 20 | 1 | 2 | 46.7 | 163 | 64 | 173 | 1202 | 25.7 | 34.1 | − 8.2 | − 7.8 |
| 4 | 21 | 1 | 2 | 47.7 | 153 | 68 | 163 | 1133 | 23.7 | 33.2 | −12.5 | −10.2 |
| 5 | 21 | 2 | 4 | 45.3 | 160 | 66 | 156 | 1084 | 23.9 | 31.4 | −15.7 | −15.1 |
| 6 | 22 | 3 | 6 | 51.0 | 157 | 77 | 178 | 1237 | 24.3 | 34.5 | − 7.0 | − 6.7 |
| 7 | 22 | 2 | 4 | 41.0 | 148 | 78 | 165 | 1146 | 27.9 | 36.7 | − 5.8 | − 0.8 |
| 8 | 22 | 2 | 4 | 46.0 | 148 | 61 | 158 | 1098 | 23.9 | 33.6 | −13.2 | − 9.1 |
| 9 | 22 | 2 | 4 | 41.0 | 154 | 62 | 156 | 1084 | 26.4 | 33.7 | −11.8 | − 8.9 |
| 10 | 23 | 2 | 4 | 49.5 | 155 | 66 | 185 | 1285 | 25.9 | 36.9 | − 1.6 | − 0.2 |
| 11 | 23 | 2 | 4 | 48.7 | 161 | 63 | 161 | 1119 | 22.9 | 31.5 | −14.6 | −14.8 |
| 12 | 23 | 2 | 4 | 43.7 | 158 | 65 | 154 | 1070 | 24.5 | 31.8 | −14.9 | −14.0 |
| 13 | 24 | 2 | 4 | 50.2 | 158 | 58 | 171 | 1188 | 23.7 | 33.2 | − 9.6 | −10.2 |
| 14 | 24 | 3 | 6 | 44.7 | 159 | 60 | 166 | 1153 | 25.8 | 34.6 | − 8.8 | − 6.4 |
| 15 | 24 | 2 | 4 | 42.7 | 149 | 59 | 140 | 895 | 20.9 | 28.2 | −26.9 | −23.7 |
| 16 | 26 | 2 | 4 | 42.1 | 149 | 64 | 154 | 1070 | 25.4 | 33.7 | −11.7 | − 8.9 |
| Average | 22.1 | | | 46.3 | 156 | 66 | 166 | 1146 | 24.7 | 33.6 | −10.6 | − 9.3 |

*The Data.* Some typical examples and the average of all the results of the determinations on 88 men are given in Table I. They indicate a lower level of basal metabolism than that for white peoples. The average deviation of the actual from the Aub-DuBois prediction standard is about 8 per cent. The significant point about these figures is that of the total of 104 subjects examined, only 16 showed a plus metabolism and of these 9 had a metabolic level of not more than 1.5 per cent. The data on 16 women are collected in Table II, showing an average level of 10 per cent. Accepting the justified claim of Benedict (1928) that the present prediction

standards for women are 5 per cent too high, it will place the degree of lowering of the basal metabolism of Filipino women at a level in substantial agreement with the degree of lowering of that of the men. Considering the grade of intelligence and the cooperative spirit of the subjects and the number of tests made on each, we feel that our data represent the true basal metabolic level of Filipinos as nearly as could be desired.

*Previous Work Among Filipinos.* So far, only two isolated observations have been made on the basal metabolism of Filipinos. Fleming (1923) in connection with his studies on the blood chemistry of Filipinos reported the respiratory metabolism of 8 male "hospital normals" using the more exact technic of Boothby and Sandiford. He found the basal metabolic level to be −5.3 per cent (Aub-DuBois), which is in close agreement with our findings, in spite of the fact that only one test was performed on each subject, and that the "hospital normals" can hardly be in a class with healthy normal people.

Sison and Ignacio (1927) made observations on 43 men and 33 women, all "hospital normals," using the same type of apparatus employed in the present work, but with the subjects sitting on a wheeled chair. Whereas with the men they found an average basal metabolism of about −7 per cent with the women it was −0.8 per cent (Aub DuBois). It is doubtful whether this figure for women represents the true state of affairs, considering that the prediction standards for women are 5 per cent too high. Sison admits that these female subjects were much more nervous than the men and many of them trembled with fear while the experiments were being performed. Table III gives the comparative results of the above named authors and our own. With the exception of Sison and Ignacio's figures for women, the metabolism levels found by three independent workers are in close agreement, namely, that the basal metabolism of the Filipinos is about 6 per cent lower than that of the whites.

*Comments.* The complexity of the factors affecting basal metabolism makes it difficult to delimit and evaluate them. That the basal metabolism of the white race is lowered as a result of long residence in a tropical climate is indicated by the work of de Almeida (1921), Hafkesbring and Borgstrom (1926) and of Sundstroem (1926). That climate has very little influence, if at all, is suggested by the findings of Eykman (1896) and of Earle (1928). In view of these apparently contradictory tendencies a little analysis of some phases of the work of each of the above named authors is of interest.

The subjects studied by de Almeida, by Hafkesbring and Borgstrom,

and by Sundstroem have one peculiarity in common; namely, that they were born and raised in the tropical or sub-tropical climate of Brazil, New Orleans and Queensland respectively. Eykman worked among Europeans, presumably Dutch, living in Java. This country, unlike Brazil or Queensland is populated by aborigines ruled by white men. In this type of tropical colony not many whites are born and live and die without spending a good part of their lives in the homeland. Relatively speaking, there are not many Dutchmen who are born, live and die in Java and much

TABLE III

BASAL METABOLISM OF FILIPINOS BY DIFFERENT WORKERS

| Authors | Apparatus Used | Type of Subjects | Men | | Women | |
|---|---|---|---|---|---|---|
| | | | No. | Per cent Deviation Aub-DuBois Standard | No. | Per cent Deviation Aub-DuBois Standard |
| Fleming | Tissot spirometer | Hospital normals | 8 | −5.3 | — | — |
| Sison and Ignacio | Benedict-Roth Portable | Hospital normals | 43 | −7.4 | 33 | −0.8 |
| Ocampo, Cordero and Concepcion | Benedict-Roth Portable | Normal students Professors Laboratory helpers | 88 | −7.8 | 16 | −9.3 |

less was this true at the time of Eykman's experiments. We are inclined to believe that Eykman's subjects were of only a few years' residence in Java.

Although Earle (1928) states in his paper that "the average basal metabolism of Westerners living in China, and particularly in the subtropical regions of China, is lower than that of Westerners living in a temperate climate," we believe that the differences he found in his rather limited series are too small to be significant. Thus in his Series I, Table 3, page 73, the average basal metabolism of Britishers living in South China is −5 per cent (Aub-DuBois). In Series III of the same table, the average for 17 British soldiers in Hongkong is −3 per cent (Aub-DuBois). On the other hand, in Series II, Table 4, page 74, the average for 23 Britishers in London is −2.5 per cent (Aub-DuBois). More important still is the fact that although the averages in all the series tend to show a negative value, the scattering of plus and minus values is rather extensive. Those familiar

with British colonials in colonies not populated by British descendants will agree that there are greater probabilities that Earle's subjects were not born and raised in China.

In view of the foregoing considerations we are led to infer that in those observations in which the basal metabolism of the whites appears to be lowered by tropical residence, we are dealing with groups of white peoples that have been born and raised in the tropics; and that in those in which no appreciable difference is noted, the subjects have had only a few months

TABLE IV

INFLUENCE OF RESIDENCE IN A COLD CLIMATE ON THE BASAL METABOLISM OF TWO FILIPINOS

| Subject | O consumed per min. (cc.) | Date of Test | Place |
|---|---|---|---|
| E. A. | 232 | Dec. 26, 1926 | Chicago |
| (No. 86, Table I) | 233 | Dec. 28, 1926 | " |
| | 195 | June 24, 1929 | Manila |
| | 203 | June 29, 1929 | " |
| N. C. | 158 | April 6, 1926 | Chicago |
| (No. 79, Table I) | 170 | May 4, 1926 | " |
| | 159 | Nov. 2, 1928 | Manila |
| | 163 | Nov. 9, 1928 | " |

or years of tropical residence. Support of this view is furnished by the observations of Earle (1928) and of McLeod, Crofts and Benedict (1925) on the metabolism of the Chinese which is consistently low whether the subjects are those born and raised in China or those of a few years' residence in America. The observations of Williams and Benedict (1928) indicate that a residence of 8 months in a sub-tropical environment did not materially alter the basal metabolism of 4 American subjects. Two of our own subjects in the present series have had their basal metabolism measured in the winter and spring in Chicago after over a year's residence there and these can be compared with measurements made after almost a year's readaptation to tropical environment. The results are given in Table IV. N. C., subject No. 79, showed no appreciable change in oxygen consumption. That of E. A., subject No. 86, diminished considerably, but this is without much significance. He has increased in adiposity since his return to Manila.

It is probable that climate is in the last analysis responsible for the different levels of metabolism of different racial groups, but that such an influence becomes manifest after generations of adaptation, or at any rate after a longer period than the span of an ordinary adult life-time.

We feel that the inclusion for purposes of scientific discussion of all European and American nationals under the general term "white race" is not satisfactory. Some of the works on the influence of tropical climate on basal metabolism have been done in Brazil, Cuba, New Orleans, etc.,

TABLE V

BASAL METABOLISM OF "ORIENTALS"

| Nationality of subjects | Total No. of subjects | Authors | Average Basal Metabolism by European Standard per cent |
|---|---|---|---|
| Filipino | 188 | Fleming<br>Sison and Ignacio<br>Ocampo, Cordero and Concepcion | −6.1 |
| Chinese | 166 | Earle<br>Necheles and Shen<br>Sloan | −8.0 |
| Japanese | 173 | Takahira<br>Okada, et al | −2.6 |

regions which have been settled originally by peoples from Southern Europe. The accepted standards of basal metabolism have been taken from peoples of Northern Europe and Northern United States. Is it not possible that the low metabolism of "whites" in the non-European territories referred to is due not merely to adaptation in these tropical or sub-tropical regions but primarily to an originally low metabolism, the result of generations of adaptation to warm Southern Europe? We are unable to find in the literature metabolism determinations among Spaniards and Italians in Europe.

Likewise, the general classification of all inhabitants of the Far East under the general term "Oriental" is not satisfactory. The findings of Earle on Chinese and ours on the Filipinos show the metabolism of these peoples to be lower than that of the Japanese. Table V gives the average metabolism levels of these three groups of "Orientals." The observations

of Takahira (1925) on 120 subjects, and of Okada (1926) on 53 subjects, are quite authoritative on the basal metabolism of the Japanese. That of MacLeod, Crofts and Benedict (1925) included only 2 Japanese of long residence in America, but owing to the indiscriminate use of the term "Oriental" the low results obtained in the series consisting mostly of Chinese subjects have been widely quoted in the literature as an opinion tending to affect the findings of the Japanese workers. There is need in this type of scientific discussion of distinguishing different types of "white races" as well as of "Orientals."

## Summary

1. The metabolism of 88 men and 16 women was determined by the Benedict-Roth portable metabolism apparatus and found to be −7.8 and −9.3 per cent respectively lower than the Aub-DuBois standards.

2. A simple method for testing for leaks in the portable respiration apparatus is described.

3. Observations of basal metabolism on 2 Filipinos while in the temperate climate show no appreciable difference from those made in the Philippines.

4. The bearing of the present report on the question of racial and climatic factors in metabolism is discussed.

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# A CHEMICAL AND IMMUNOLOGICAL STUDY OF EGG PROTEIN OBTAINED UNDER RESTRICTED DIETS

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Received for Publication—March 3, 1930

WHILE it is known that the yolk and white of a hen's egg represent the secretions elaborated by the ovary and oviduct respectively, the process of the formation of these secretions from available material is practically unknown. There is, however, ample reason to believe that certain metabolic products as represented by eggs are quite largely modified, particularly in the chemical sense, by the kind of food ingested. Furthermore, the production of eggs would cease entirely if the complement and quality of substances necessary for the formation of egg-complexes were not satisfactory.

Modification of these egg-complexes by the ration consumed has been shown by Sherwood (1) and Bethke, Kennard and Sassaman (2). Plimmer and co-workers (3) refer production of eggs and their hatchability to the vitamin B content of the foodstuffs; while Pollard and Carr (4), restricting pigeons to a single kind of grain, correlated the different grains used with chemical composition of eggs produced. Hektoen and Cole (5,6) conclude that conalbumin is carried over unchanged from the blood of the hen to the egg white.

This identity was established by immuno-chemical methods, which were also used by Dale and Hartley (7) and Dakin and Dale (8) to show antigenic differences between the crystallized albumins from hen egg and duck egg. They conclude that stereo-chemical structure of a protein molecule is, at least, an important factor in its antigenic specificity. Taking the reverse of this and applying the anaphylaxis reaction, differences in egg protein structure were studied and correlated with chemical differences manifested by nitrogen distribution schemata. A study was also made of the fertility of the eggs obtained from pigeons restricted to one of a variety of cereal grains.

## EXPERIMENTAL

To obtain eggs for study pigeons were used inasmuch as they are easily handled, consume less food than would chickens, and adjust themselves readily to close quarters. The birds, common pigeons, were paired, dusted

with sodium fluoride powder, and placed in wire cages about three feet in each dimension—one pair of birds to a cage.

The quarters were warm, light and airy, although only skylight was available. The pigeons were all well supplied with grit, charcoal, oyster shell, salt and tap water; the salt being sprinkled over the mineral mixture from which the birds fed *ad lib.* At the outset wheat was doled out to all birds until the first egg had been laid at which time the grain was changed to one of the following; corn, oats, kafir, hemp, soy beans, peas, cotton seed meal powdered skim milk, barley, rye, or wheat. The eggs obtained after 3 to 4 months on the restricted rations were used for subsequent analytical and immunological proceedings.

In Table I.

a. *Total weight* refers to average weight of entire egg, shell included.

b. *Weight of material* is an average weight of undried egg exclusive of shell and shell membrane:

c. *Dry weight* is an average weight of the egg material after drying in vacuo at 40° C.

d. *Grain consumption* is an average figure per bird per day.

In this table there is apparently a direct relationship between the amount of grain consumed, total weight of egg and dry weight—with the exception of rye, which seems to produce an egg containing more water. For four weeks in the first part of the experiment cod-liver oil was added to the amount of 1 per cent of the ration. This addition was made in view of the generally accepted idea that cod-liver oil tends to give an egg a thicker shell, hence, is a safety factor in preventing the breaking of eggs. However, there was neither noticeable thickening of shells, nor elimination of broken eggs. Since the pigeons would not eat the oil-covered grains nearly as well as the untreated food the inclusion of cod-liver oil was dropped with the exception of one lot on kafir, marked with an asterisk in Table I.

Though there is considerable variation in the egg-laying capacity of different pigeons on the same food, average figures for the number of days per egg rate the grains in the following order of decreasing efficiency in egg production: soybeans (6.4), oats (6.4), kafir (6.6), hemp (6.8), wheat (8.6), corn (10.0), barley (11.6), rye (22.3), Canada field peas (68). Cottonseed meal and powdered skim milk did not yield any eggs at all. Pigeons feeding on whole oats, consisting largely of fibrous hulls, proved to be non-productive, but changing the ration to steel cut hulled oats produced a marked benefit with good yield of eggs. Robinson (9)

TABLE I

GRAIN CONSUMPTION, EGG-WEIGHTS AND EGG PRODUCTION OF PIGEONS FOR THE VARIOUS RATIONS

| Grain | Total wt. gms. | Material wt. gms. | Dry wt. gms. | No. of Eggs | | | | No. of days per egg | | | | Grain consumption gms. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Lot 1 | Lot 2 | Lot 3 | Ave. | Lot 1 | Lot 2 | Lot 3 | Ave. | |
| Wheat..... | 15.28 | 13.55 | 2.90 | 11 | 10 | 17 | 12.67 | 12.2 | 7.8 | 5.94 | 8.6 | 19.10 |
| Corn..... | 18.29 | 16.35 | 3.24 | 11 | 13 | | 12 | 12.2 | 7.76 | | 10.0 | 25.15 |
| Kafir..... | 14.79 | 13.36 | 2.65 | 26 | 11* | 12 | 16.33 | 5.2 | 8.81* | 6.73 | 6.6 | 18.33 |
| Barley..... | 15.31 | 13.83 | 3.00 | 13 | 10 | | 11.5 | 10.3 | 13.4 | | 11.6 | 22.00 |
| Oats..... | 19.30 | 17.29 | 3.43 | 6 | 6 | 14 | 8.67 | 5.5 | 5.5 | 7.22 | 6.4 | 29.17 |
| Rye..... | 17.53 | 15.29 | 2.75 | 6 | 3 | | 4.5 | 22.3 | 44.7 | | 22.3 | 18.25 |
| Canada field peas | | | | 1 | | | 1 | 68.0 | | | 68.0 | |
| Hemp..... | | | | 13 | | | 13 | 6.8 | | | 6.8 | 20.10 |
| Soybeans..... | | | | 14 | | | 14 | 6.4 | | | 6.4 | 21.63 |
| Cottonseed starch 1:3.... | | | | | | | | | | | | |
| Powd. skim milk starch (1:2)... | | | | | | | | | | | | |

* Cod-liver oil.

states that pigeons breed nearly the year round, thus seasonal variations played no important part in the number of eggs produced.

*Chemical Analysis of Eggs.* Eggs obtained after the pigeons had been 3 to 4 months on the restricted ration were taken for chemical analysis soon after being laid (immediately to 48 hours; in all cases kept in refrigerator until opened). The distribution of nitrogen characteristic of the proteins of the eggs was determined by the Van Slyke (10) method as modified by Plimmer and Rosedale (11,12) stannous chloride being employed as recommended by Sullivan (13) for keeping down humin formation. The shell and membrane were cut open allowing the entire contents of the egg to drop into an 800 ml. Kjeldahl flask, a rubber policeman being used for the last portions of egg white that cling to the shell, care being taken not to remove any shell membrane. Hydrolysis was effected by boiling gently on a sand bath with HCl (sp. gr. 1.115) containing 1 gm. of $SnCl_2$. Before analysis the eggs of those pigeons on hemp and soybeans were dried in a vacuum desiccator over sulfuric acid, and fat was extracted by means of petroleum ether. Analyses are summarized in Table II.

Certain divergences in nitrogen distribution are evident from Table II, for while the percentages of nitrogen in the original egg material vary but little, marked differences are noted in the nitrogen distribution. Since the significance of humin nitrogen is not exactly defined and since it is known to result otherwise than from the decomposition of tryptophane no definite inference can be drawn with regard to this fraction. Values for ammonia nitrogen are highest for soybean and hemp and lowest for rye; "kafir" eggs show highest diamino nitrogen and lowest monoamino nitrogen, while "soybean" eggs yield lowest values for diamino nitrogen and highest for mono-amino nitrogen.

From the published data of Grindley and Slater (14), Nollau (15), and Hamilton, Nevens, and Grindley (16), unquestionable differences in the protein make-up of the different grains, as evinced by nitrogen distribution, are apparent. There seems to be a tendency for the pigeon to smooth out the variations to some extent in laying down the proteins of the egg; but since even a similarity in amino-acid make-up does not preclude the possibility of spatial deviations, which play an important part in determining specificity in immunological reactions, it was deemed advisable to investigate further differences in the protein pattern of the various eggs. For this purpose a colony of white rats and also guinea pigs were sensitized with suspensions of the eggs and tested by means of the anaphylaxis reaction.

TABLE II
NITROGEN DISTRIBUTION OF EGG PROTEINS IN PERCENTAGE OF TOTAL NITROGEN

| Grain | Nitrogen in original egg per cent | Ammonia Nitrogen | Total Humin N | Di-amino Fraction | | | Mono-amino Fraction | | | Total by summation |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total N | Amino N | Non-amino N | Total N | Amino N | Non-amino N | |
| Corn | 2.00 | 6.56 | 4.74 | 38.95 | 24.48 | 14.47 | 50.65 | 47.93 | 2.72 | 100.30 |
| Corn | 2.02 | 6.90 | 3.60 | 38.46 | 23.00 | 15.46 | 50.44 | 49.07 | 1.37 | 99.40 |
| Oats | 2.08 | 8.08 | 2.92 | 35.66 | 24.25 | 11.41 | 51.59 | 49.42 | 2.17 | 98.25 |
| Oats | 2.04 | 8.03 | 4.99 | 35.51 | 24.35 | 11.16 | 51.41 | 49.03 | 2.38 | 99.93 |
| Kafir | 2.15 | 7.48 | 1.86 | 43.26 | 28.71 | 14.55 | 47.01 | 45.13 | 1.88 | 99.61 |
| Kafir | 2.19 | 6.93 | 3.24 | 42.62 | 29.33 | 13.29 | 46.20 | 44.97 | 1.23 | 98.99 |
| Wheat | 2.23 | 6.59 | 4.72 | 32.72 | 21.98 | 10.74 | 55.60 | 52.49 | 3.11 | 99.63 |
| Barley | 2.36 | 8.41 | 3.60 | 35.37 | 25.31 | 10.06 | 51.47 | 48.64 | 2.83 | 98.84 |
| Barley | 2.33 | 8.39 | 4.47 | 35.20 | 24.28 | 10.92 | 51.29 | 49.81 | 1.48 | 99.35 |
| Rye | 2.22 | 4.73 | 3.38 | 35.50 | 24.70 | 10.20 | 53.60 | 51.21 | 2.39 | 97.21 |
| Rye | 2.23 | 4.76 | 4.13 | 35.69 | 24.62 | 11.07 | 53.10 | 51.09 | 2.01 | 97.68 |
| Hemp | | 6.95* | 2.89 | 25.01 | 14.75 | 11.26 | 60.23 | 56.60 | 3.63 | 94.88 |
| Hemp | | 9.95 | 2.47 | 25.44 | 14.98 | 10.46 | 61.88 | 57.23 | 4.65 | 99.74 |
| Soybean | | 10.40 | 2.85 | 23.46 | 15.08 | 8.38 | 62.54 | 55.40 | 7.14 | 99.75 |
| Soybean | | 10.21 | 2.85 | 23.68 | 15.47 | 8.21 | 63.08 | 55.93 | 7.15 | 99.82 |

* Low value due to incomplete distillation.

TABLE III
ACTIVE SENSITIZATION OF WHITE RATS TO EGG PROTEINS

| Rat No. | Inoculum | Dates of sensitization | Amt. of 1:100 Sol. | Shock inoculum | Date of shock inj. | Weight gms. | Time after last inj. days | Amt. shock dose | Conc. | Method of shock inj. | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | cc. | | | | | cc. | | | |
| 1 | Wheat | June 26 & 28 | 5–2 | Wheat | July 15 | 150.7 | 17 | 5 | 1:10 | Intra-ven. | Dead 22 mins. |
| 2 | " | " | 2–3 | " | " | 155.7 | 17 | 3 | 1:10 | Intra-ven. | Dead 27 " |
| 3 | " | " | 2–2 | Corn | " | 124.2 | 17 | 2 | 1:10 | Intra-ven. | Dead 57 " |
| 4 | Kafir | " | 2–2 | Kafir | " | 162.5 | 17 | 5 | 1:100 | Intra-per. | Severe |
| 5 | " | " | 2–2 | " | " | 145. | 17 | 1 | 1:10 | Intra-per. | Dead in 1 hr. 10 mins. |
| 6 | " | " | 2–2 | Corn | " | 153.8 | 17 | 5 | 1:100 | Intra-per. | Moderate |
| 7 | Corn | " | 2–2 | " | July 16 | 164.7 | 18 | 1 | 1:10 | Intra-ven. | Dead in 2 hrs. 35 mins. |
| 8 | " | " | 2–2 | Wheat | " | 181. | 18 | 3 | 1:100 | Intra-ven. | Severe |
| 9 | " | " | 2–2 | Corn | " | 200.5 | 18 | 5 | 1:100 | Intra-per. | Slight |
| 10 | Barley | " | 2–2 | Barley | " | 171. | 18 | 5 | 1:100 | Intra-per. | Moderate |
| 11 | " | " | 2–2 | Kafir | " | 197.1 | 18 | 1 | 1:10 | Intra-ven. | Moderate |
| 12 | Oats | June 27 & 29 | 2–2 | Oats | July 16 | 149.3 | 17 | 5 | 1:100 | Intra-per. | Severe |
| 13 | " | " | 2–2 | Barley | " | 169.4 | 17 | 5 | 1:100 | Intra-per. | Moderate |
| 14 | " | " | 2–2 | Wheat | " | 160. | 17 | 1 | 1:10 | Intra-ven. | Severe |
| 15 | Rye | " | 2–2 | Rye | " | 197. | 17 | 2 | 1:10 | Intra-ven. | Dead in 1 hr. 15 min. |
| 16 | " | " | 2–2 | " | " | 179.3 | 17 | 2 | 1:10 | Intra-ven. | Dead in 1 hr. 5 mins. |
| 17 | Diluent salt & glycerin | " | 2–2 | Diluent salt & glycerin | July 15 | 159.4 | 16 | 5 | | Intra-per. | None |
| 18 | None | — | — | " | " | 161. | 16 | 2 | | Intra-ven. | None |
| 19 | None | — | — | Wheat | " | 100.6 | 16 | 2 | 1:10 | Intra-ven. | Slight |
| 20 | " | — | — | Kafir | " | 193.7 | 16 | 5 | 1:100 | Intra-ven. | None |

TABLE III (*continued*)

| Rat No. | Inoculum | Dates of sensitization | Amt. of 1:100 Sol. | Shock inoculum | Date of shock inj. | Weight gms. | Time after last inj. days | Amt. shock dose | Conc. | Method of shock inj. | Symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | Wheat | July 3, & 5 | 2–3 | Wheat | July 20 | 154.5 | 15 | 5 | 1:100 | Intra-per. | Slight |
| 22 | " | " | 2–2 | — | — | — | — | — | — | — | Found dead July 8. |
| 23 | Kafir | " | 2–2 | Rye | " | 148.5 | 15 | 2 | 1:100 | Intra-ven. | Dead in 58 mins. |
| 24 | Kafir | " | 2–2 | Corn | July 21 | 141.1 | 16 | 2 | 1:100 | Intra-ven. | Dead in 1 hr. 5 mins. |
| 25 | Barley | " | 2–2 | Oats | " | 96.5 | 16 | 5 | 1:100 | Intra-per. | Moderate |
| 26 | " | " | 2–2 | Rye | " | 94.4 | 16 | 5 | 1:100 | Intra-per. | Slight |
| 27 | " | " | 2–2 | Wheat | " | 92.4 | 16 | 5 | 1:100 | Intra-per. | Severe |
| 28 | " | " | 2–2 | Corn | " | 96.1 | 16 | 1 | 1:10 | Intra-ven. | Slight |
| 29 | Rye | " | 2–2 | Kafir | " | 93.1 | 16 | 5 | 1:100 | Intra-per. | Slight |
| 30 | " | " | 2–2 | Rye | " | 84.6 | 16 | 5 | 1:100 | Intra-per. | Moderate |
| 31 | " | " | 2–2 | Wheat | " | 88.5 | 16 | 2 | 1:100 | Intra-ven. | Slight |
| 32 | Oats | " | 2–2 | Corn | " | 87. | 16 | 2 | 1:10 | Intra-ven. | Slight to moderate |
| 33 | " | " | 2–2 | Kafir | " | 76. | 16 | 5 | 1:100 | Intra-per. | None |
| 34 | " | " | 2–2 | Rye | " | 86.5 | 16 | 1 | 1:10 | Intra-ven. | Moderate |
| 35 | Corn | " | 2–2 | " | " | 94.8 | 16 | 2 | 1:10 | Intra-ven. | Dead in 1 hr. 25 mins. |
| 36 | " | " | 2–2 | Kafir | " | 96.5 | 16 | 5 | 1:100 | Intra-per. | Severe |
| 37 | " | " | 2–2 | Barley | " | 91. | 16 | 2 | 1:100 | Intra-ven. | Moderate |

"Severe symptons" indicates very severe prostrations, dyspnea and weakness for at least three hours.
"Moderate symptons" indicates very severe prostration for one to three hours.
"Slight symptoms" indicates weakness and prostration for not more than one hour.

*Immunological Relationships of the Egg Proteins.* Although some investigators have reported the insusceptibility of white rats to sensitization, others have been able to obtain positive anaphylactic reactions in mice and rats, notably Parker and Parker (17) who were able to show active and passive sensitization by both *in vitro* and isolated-uterine-strip methods.

For active sensitization a fresh egg was diluted 1:10 with a solution composed of 66–2/3 volumes of glycerin and 33–1/3 volumes of a solution containing 2.5 gms. NaCl, 2.7 gms. $NaHCO_3$, and 5 gms. phenol per liter. For inoculation this stock suspension was further diluted 1:10 with physiologic salt solution. Precautions were taken to prevent contamination and the stock solution was preserved in the refrigerator. Sensitizing injections were made intraperitoneally, and whenever a shock injection was made intravenously the femoral vein was used. Table III, shows the results.

A protection test was applied to some of the rats as a means of checking the reaction, Table IV. In this test the rats were allowed to rest 1 to 2 days after administration of the shock dose and then were given an injection of the homologous protein. If the animal gave no further reaction upon the latter inoculation, protection was complete. In the event of incomplete saturation with the heterologous protein, protection was classed as partial, slight, or nearly complete, indicative of the degree of further intoxication upon injection.

Characteristic symptoms of anaphylactic shock in the rat as described by Parker and Parker (17) were observed, as well as rapid muscular twitchings of the injected leg about the site of injection in those animals showing severe reactions.

It is evident that while white rats have been shown to be actively sensitized to egg protein, there is only a broad quantitative indication of individuality among the various proteins used. Intoxication with the homologous protein usually resulted in a more severe reaction than injection of a heterologous protein.

*Anaphylaxis Tests Using Sensitized Guinea Pigs.* Guinea pigs were sensitized actively using as inocula suspensions of egg white, carefully separated from yolk, diluted 1:100 with physiologic saline. All sensitizing injections were made intraperitoneally in 2 ml. amounts on the following dates: March 24, 27, and 30. Intoxicating inoculations were made with the original preserved solution of 1:10 dilution. Table V summarizes the findings. The names in columns two and three refer to the grains with which the pigeons were provided. Post-mortem examinations revealed in every case of fatal reaction death due to anaphylactic shock.

TABLE IV
PROTECTION TESTS USING HOMOLOGOUS PROTEINS

| Rat. no. | Sensitizing inoculum | Days incubation | Shock inoculum | Symptoms | Test inoculum | Amt. cc. | Conc. | Method of test inj. | Days between shock & test inj. | Protection |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Oats | 17 | Wheat | Severe | Oats | 5 | 1:100 | Intra-per. | 2 | Nearly complete |
| 17 | Salt & glycerin diluent | 16 | Salt & glycerin diluent | None | Corn | 1 | 1:10 | Intra-ven. | 1 | Dead in 1 hr. 17 mins. |
| 18 | None | — | Salt & glycerin diluent | None | Oats | 2 | 1:10 | Intra-ven. | 1 | No reaction |
| 19 | None | — | Wheat | Slight | Wheat | 1 | 1:10 | Intra-ven. | 1 | No reaction |
| 20 | None | — | Kafir | None | Kafir | 5 | 1:100 | Intra-per. | 1 | No reaction |
| 21 | Wheat | 15 | Wheat | Slight | Corn | 5 | 1:100 | Intra-per. | 2 | Partial |
| 22 | Barley | 16 | Oats | Moderate | Barley | 2 | 1:100 | Intra-ven. | 2 | Partial |
| 33 | Oats | 16 | Kafir | None | Oats | 1 | 1:10 | Intra-ven. | 1 | Complete |
| 36 | Corn | 16 | Kafir | Severe | Corn | 5 | 1:100 | Intra-ven. | 2 | Nearly complete |
| 37 | Corn | 16 | Barley | Moderate | Corn | 1 | 1:10 | Intra-ven. | 2 | Complete |

Here again appears an interrelationship between the proteins of the various eggs, but quantitative differences in the intensity of the anaphylactic reaction are quite evident. In almost every case death, accompanied by typical anaphylactic symptoms, ensued more rapidly with the homologous protein than with the heterologous protein.

TABLE V

ACTIVE SENSITIZATION OF GUINEA PIGS WITH EGG PROTEINS

| Guinea pig No. | Sensitizing inoculum | Shock inoculum | Date and amount 1:10 | Method of injection | Results |
|---|---|---|---|---|---|
| | | | cc. | | |
| 1 | Control | Wheat | 5/14 2 | Intraven. | Negative |
| 2 | " | Hemp | 4 | Intraper. | " |
| 3 | " | Corn | 2 | Intraven. | " |
| 4 | Wheat | Wheat | 2 | Intraper. | Dead in 25 minutes. |
| 5 | " | Corn | 1 | Intraven. | Dead in 25 minutes. |
| 6 | " | Kafir | 3 | Intraper. | Dead in 34 minutes. |
| 7 | Wheat Yolk* | Wheat | 1 | Intraven. | Moderate symptoms. |
| 8 | Wheat | Hemp | 2 | Intraper. | Dead in 8 minutes. |
| 9 | Corn | Corn | 2 | Intraven. | Dead in 3 minutes. |
| 10 | Corn Yolk* | Corn | 1 | Intraven. | Negative. |
| 11 | Corn | Oats | 2 | Intraper. | Severe symptoms. |
| 12 | Corn | Kafir | 2 | Intraven. | Dead in 20 minutes. |
| 13 | Oats | Oats | 3 | Intraper. | Dead in 38 minutes. |
| 14 | Oats | Hemp | 2 | Intraper. | Dead in 44 minutes. |
| 15 | Kafir | Wheat | 2 | Intraper. | Dead in 30 minutes. |
| 16 | Kafir | Corn | 2 | Intraper. | Dead in 25 minutes. |
| 17 | Hemp | Soybeans | 2 | Intraper. | Dead in 24 minutes. |
| 18 | Hemp | Wheat | 1 | Intraven. | Found dead 18 hours later. |
| 19 | Hemp | Kafir | 2 | Intraper. | Dead in 25 minutes. |
| 20 | Soybeans | Oats | 2 | Intraper. | Dead in 30 minutes. |
| 21 | Soybeans | Wheat | 1 | Intraven. | Dead in 30 seconds. |
| 22 | Soybeans | Soybean | 2 | Intraper. | Dead in 9 minutes. |

* Yolks carefully separated and washed free from white, suspended in the glycerin—$NaHCO_3$ medium; diluted 1:100.

*Fertility and Hatchability of Incubated Eggs.* Needham (18) expressed the view "that in the first 10–14 days chemical change is limited to those compounds which are required principally for structural purposes, while towards the end of incubation the opening up of functional activities causes marked and profound chemical changes of other kinds." The suitability of the proteins contained in the eggs derived from the different grains as a source of meeting both of these ends was investigated in an introductory manner.

While considerable depends upon the bird itself as a reproducer, there are other factors contributing to the fertility and hatchability of its eggs such as protein of food supply, vitamins, mineral supply, and even environment. Eggs obtained from the pigeons on the different grains were transferred to an incubator as soon as possible after beng laid, the time varying from 5 minutes to 24 hours, allowed to hatch, or given such time in the incubator as should have resulted in hatching, and then opened. Other eggs were opened after 72 hours incubation, the embryos removed, mounted, and photomicrographs prepared. Those from hemp and corn are shown for the sake of contrast. Plates 1 and 2.

PLATE 1

*A*. Grain: Hemp—Egg. No. of hours incubated: 72. Remarks: About 72 hrs. development.

PLATE 2

*B*. Grain: Corn—Egg. No. of hours incubated: 72. Remarks: 30 hours development.

Soybeans, hemp, wheat, and kafir all have a favorable influence upon the development of embryos, whereas embryos from corn and oats were usually many hours behind those derived from other grains. While it may be true that vitamins play a most significant rôle in ultimate hatching, and particularly in rearing, at which time the young squab may need the complement of vitamins, egg production seems to be singularly a property bestowed by the proteins of the grain. This view may be further examined by a study of the early development of the embryo for, while no definite proof can be given *pro* or *con*, the tacit assumption is made that the embryo in the first few days of development does not require vitamins. Progress should then be manifested almost solely as a function of the proteins laid down in the egg.

## SUMMARY

Pigeons were kept under controlled conditions with rations limited to a single kind of grain supplemented with grit, oyster shell, charcoal, salt

and tap water. The birds maintained weight, health, vigor, and egg production, except in the cases noted, for a period of about one hundred and fifty days.

Representative eggs were subjected to a modified Van Slyke procedure for the determination of nitrogen distribution and showed differences in the chemical make-up of their proteins. Ammonia nitrogen showed the largest deviations represented by 4.75 per cent of total nitrogen for "rye" eggs and 8.40 per cent for "barley" egg protein. Total nitrogen content of the egg material was fairly constant showing slight absolute differences. Mono-amino nitrogen was higher in the eggs obtained from the feeding of hemp, soybeans, and wheat, while "kafir" eggs showed a higher diamino nitrogen content. Corn and kafir yielded eggs with the highest non-amino nitrogen in the basic fraction.

It was possible to produce anaphylactic shock in white rats and guinea pigs through which means quantitative differences were indicated in the immunological relationships between the egg proteins from the various sources suggesting dissimilar chemical composition.

Incubation of a number of eggs indicates the importance of the protein present in the egg in the development of the embryo, both at the beginning and the end of incubation. Initial progress, shown by a series of photomicrographs, implies a beneficence of the proteins supplied to the egg by hemp, soybeans, and wheat, whereas corn and oat embryos developed much more slowly, indicating a poorer quality of protein.

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# THE VITAMIN B AND G CONTENTS OF CERTAIN YEAST SAMPLES*

*By*

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Received for Publication—March 4, 1930

A STUDY was recently made to determine the vitamin B and vitamin G content of several yeast samples that were to be used for vitamin research problems in this laboratory. Although the methods of determining the potency of the yeasts were very different from the ones employed by Scheunert and Schieblich[1] in their comparison of bakers' yeast and brewers' yeast, the results are quite in agreement with those of the German investigators and indicate that the vitamin B content of different samples of yeast may vary much more widely than the vitamin G content.

The quantitative methods employed in the present work and the results obtained are presented in some detail as of possible interest to those concerned with the vitamin content of this product.

Included in this investigation were samples obtained from different sources in this country or Canada. Among them were two samples of brewers' yeast, one sample of an Eastern product labeled, "dry powdered yeast," a sample of fresh starch-free bakers' yeast and a "pure dehydrated yeast" obtained from a Mid -Western manufacturer of this material. One of the samples of brewers' yeast was from a Canadian brewery, the other from a New York City producer. The Canadian sample was "top yeast" that had been dried at the plant in steam heated drums and then ground to a rather coarse powder, the New York sample a fresh moist sample of "bottom yeast." It was necessary to dry the latter as well as the fresh starch-free bakers' yeast in the laboratory before use. This was accomplished by first passing each material through a sieve and then spreading it in thin layers and exposing to a current of air at room temperature for a period of 48 hours.

The methods employed in determining the vitamin B($B_1$) and vitamin G($B_2$) content of the yeast samples were those recently developed by

* Published as Contribution No. 639 from the Department of Chemistry, Columbia University, New York.

[1] Scheunert, Arthur, and Schieblich, Martin, *Biochem. Zeitschr.*, CCXII, 1929, 80.

Chase[2] and Bourquin[3] respectively. These methods, in brief, involve the feeding to young healthy albino rats at weaning age a diet that is adequate except for the vitamin to be studied and determining the effect of added supplements of the material being tested on the animals' health and body weight. Since both the antineuritic vitamin and vitamin G($B_2$) are required for growth, rats confined to a diet lacking either of these vitamins for a comparatively short time lose weight, while those receiving in addition to the deficient diets supplements containing the vitamin, grow in response to those additions in proportion to the amounts of the vitamin furnished. The weight of the experimental animal is therefore taken as the chief criterion for the measurement of the vitamin potency of the various samples investigated.

*The Vitamin B ($B_1$) Content of the Yeast Samples.* The basal diet used by Chase[2], which was the one adopted for use in this study, consists of; extracted casein, 18, autoclaved yeast* 15, cornstarch 53, butter fat 8, cod liver oil 2, Osborne and Mendel salt mixture 4 per cent. As was found by Chase, our animals when placed on this diet at 28 to 29 days of age gained slightly in weight for a short time. Apparently there was some antineuritic vitamin stored in the bodies of the animals which enabled them to grow on the deficient diet until this surplus vitamin in the body tissues became depleted.

To obviate any possible effect of this preliminary growth on the results of feeding vitamin B in the test food, the animals were fed the basal diet only, until frequent weighings showed that they were no longer gaining in weight. They were then placed in individual cages with raised screen bottoms and the experimental feeding of the yeast samples begun. The yeast was fed as daily supplements to the basal diet. A careful record was kept of the food consumed by each individual animal and weighings of the animals were made at weekly intervals throughout the experimental period of 8 weeks.

The result of feeding the different yeast samples on the growth of the animals prepared as described above is shown by the average or individual growth curves in Figure 1. The Canadian and New York samples of brewers' yeast were definitely more potent as regards their vitamin B($B_1$)

[2] Chase, E. F., Columbia Dissertation, 1928.

[3] Bourquin, A., Columbia Dissertation, 1929.

* 400 grams of bakers' yeast were thoroughly wetted with 500 cc. of N/10 NaOH and autoclaved for 6 hours at 15 pounds pressure. Subsequently an amount of N/10 HCl, equivalent to the alkali used, was added, the material dried at room temperature and finely ground.

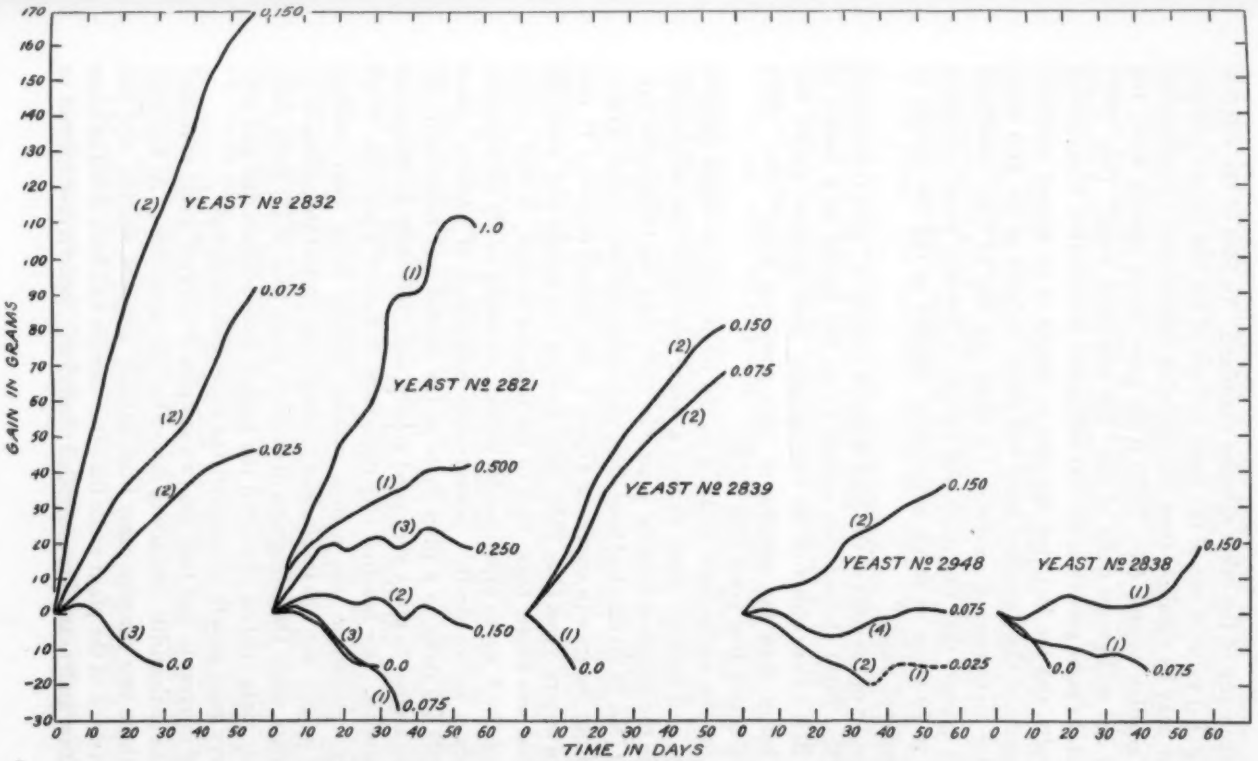


FIG. 1. A STUDY OF THE VITAMIN B ($B_1$) CONTENT OF VARIOUS YEAST SAMPLES

Growth curves of albino rats fed the yeast samples to be tested. Yeast 2832 = Brewers' yeast from a Canadian brewery. 2821 = Eastern sample of "dry powdered yeast." 2839 = Brewers' yeast from a New York producer. Sample dried in laboratory at room temperature. 2948 = Mid-Western sample of "pure dehydrated yeast." 2838 = Starch-free bakers' yeast dried in laboratory at room temperature.

Number on curve is the number of animals represented therein. Number at end of curve is the amount in grams of yeast fed daily as supplement to the deficient basal diet.

content than any of the other samples examined. An idea of the relative vitamin B($B_1$) value of one of the more and one of the less potent samples examined may be obtained from the following comparison; 25 milligrams of the Canadian sample (No. 2832) daily gave better growth when fed than did 250 milligrams daily of the "dry powdered yeast" (No. 2821) and about the same growth as did 500 milligrams of the latter. Considering these results, and the fact that the gain in weight of an animal receiving 75 milligrams of the Canadian yeast was nearly as good as the gain made by a litter mate control receiving 1.0 gram per day of "dry powdered yeast," it appears that this particular sample of brewers' yeast contained from 10 to 20 times as much antineuritic vitamin as did the sample of "dry powdered yeast."

The results of the tests of the Mid-Western sample of "pure dehydrated yeast" (No. 2948) showed this sample to be less potent as a source of vitamin B($B_1$) than either of the two brewers' yeast samples tested, but slightly better than the samples of "dry powdered yeast" (No. 2821) or bakers' starch-free yeast (No. 2838).

Tests for the antineuritic vitamin in the laboratory air-dried samples of bakers' and brewers' yeast showed a larger amount of the antineuritic vitamin in the sample of brewers' yeast (No. 2839) than was found in the bakers' yeast (No. 2838) that had been dried under identical conditions. As may be seen from the growth curves in Figure 1, one animal receiving 75 milligrams of bakers' yeast (No. 2838) daily steadily lost weight and, although its survival was longer than that of the negative control, it died before the end of the 8 weeks experimental period, becoming badly polyneuritic several days before death. Seventy-five milligrams of brewers' yeast (No. 2839) fed daily to a litter mate of the animal just mentioned, enabled this animal to make very good growth and there were no symptoms of polyneuritis. One hundred fifty milligrams of bakers' yeast (No. 2838) fed daily resulted in one animal making slightly more than maintenance of weight, but was insufficient to completely protect the animal from polyneuritis, while 150 milligrams of brewers' yeast (No. 2839) given daily to a litter mate control protected the animal from polyneuritis and produced very good growth throughout the experimental period.

It is of interest to find that the two samples of brewers' yeast examined showed an antineuritic potency of somewhat the same order. As has been stated, these two samples came from entirely different sources, and one had been dried at the plant while the other sample had been dried at room temperature under carefully controlled conditions. Again it is of interest to

find that the commercial sample of "dry powdered yeast" shows approximately the same antineuritic potency as the bakers' starch-free yeast that had been carefully dried in the laboratory. Although vitamin B ($B_1$) is considered fairly heat labile, it would appear from these results that the smaller amounts of vitamin B ($B_1$) in certain of the samples were not due wholly to a destruction of the vitamin incident to the drying of the yeast samples.

*The Vitamin G ($B_2$) Content of the Yeast Samples.* Employing essentially the same method developed by Bourquin[3] for determining the vitamin G ($B_2$) content of a food, tests have been made to determine the vitamin G potency of the same samples of yeast that are described in the first part of this paper. The basal diet used in our work consists of the following; extracted casein 15, whole wheat 20 (in some cases an 80 per cent alcoholic extract of 60 grams of whole wheat dried on cornstarch was employed), cornstarch 51, Osborne and Mendel salt mixture 4, butter fat 8, and cod liver oil 2 per cent.

This diet, when fed to young albino rats, has been found to enable the animals to make approximately normal growth when the diet is supplemented with a sufficient amount of vitamin G. Although the whole wheat or wheat extract which serves as the source of the antineuritic vitamin in this basal diet contains traces of vitamin G ($B_2$), the amount is so small that the weight of negative controls, or those fed the basal diet only, remains at a low level and the animals, if they live sufficiently long, develop many of the pellagra-like symptoms that Goldberger and Lillie[4] have described as occurring in their experimental animals.

As in the tests for the antineuritic vitamin, when rats at weaning age are placed on this basal diet, which is adequate but for vitamin G, they grow slightly for about two weeks. The body weight, which at the end of this period may be 12 to 15 grams higher than the weight at 28 days of age, then remains stationary or declines unless the vitamin is supplied. When two or three successive weighings made on alternate days show that the animals on the basal diet have ceased to gain in weight, they are then fed the test material in daily weighed portions as supplements to the basal diet. Any improvement in growth of the experimental animal, receiving the test food, over those receiving the deficient diet only, is attributed to vitamin G, and the weight gains serve as a measure of the relative amount of the vitamin supplied.

The results of the tests for vitamin G are shown in Figure 2. A relatively

[4] Goldberger, Joseph, and Lillie, R. D., *U. S. Public Health Reports*, 1926, XLI, 1025.

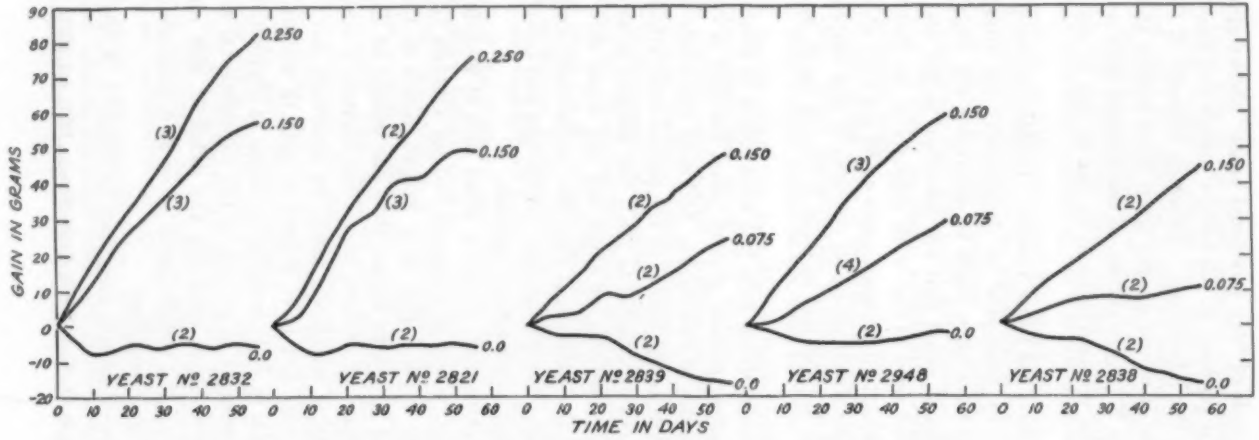


FIG. 2. A STUDY OF THE VITAMIN G ($B_1$) CONTENT OF VARIOUS YEAST SAMPLES

Growth curves of albino rats fed the yeast samples to be tested.

Yeast 2832 = Brewers' yeast from a Canadian brewery.
" 2821 = Eastern sample of "dry powdered yeast."
" 2839 = Brewers' yeast from a New York producer; sample dried in laboratory at room temperature.
" 2948 = Mid-Western sample of "pure dehydrated yeast."
" 2838 = Starch-free bakers' yeast dried in laboratory at room temperature.

Number on curve is the number of animals represented therein. Number at end of curve is the amount in grams of yeast fed daily as supplement to the deficient basal diet.

uniform amount of vitamin G was found in all of the samples examined. Apparently 75 to 150 milligrams daily of any one of the five samples of yeast would allow the experimental animals on the vitamin G-deficient diet to make unit growth (an average gain of 3 grams per week) over the experimental period of eight weeks.

Comparing the results plotted in Figures 1 and 2, we find that the Canadian and New York samples of brewers' yeast (No. 2832 and No. 2839) contained relatively more of the antineuritic vitamin than of the heat-stable factor vitamin G; or vitamin G would be considered the first limiting factor in both samples. In the case of the Canadian sample, for example, it required 25 milligrams of the yeast to produce a certain rate of growth when the test for the antineuritic vitamin was made, while 150 milligrams were necessary to give approximately this same rate of growth when the amount of vitamin G present was being measured. As regards vitamin B and G in the remaining three samples studied, vitamin B would be the first limiting factor in each case.

Since yeast is frequently used in experimental feeding work as a source of vitamins B($B_1$) and G ($B_2$), the need for insuring the presence of both factors in sufficient amounts is evident. Results of vitamin A work could easily be vitiated if the yeast used for supplying two of the vitamins required in the basal diet were inadequate either in quality or quantity.

## Summary

Quantitative tests for vitamins B ($B_1$) and G ($B_2$) in five samples of yeast showed that the concentration of vitamin B varied considerably (at least 10-fold) in the different samples, while the vitamin G ($B_2$) content of the five samples was relatively uniform. Of the two vitamins the antineuritic vitamin was the limiting factor in three of the samples while vitamin G was the limiting factor in the two samples of brewers' yeast examined.

# A STUDY OF THE ABSORPTION AND RETENTION OF VITAMIN A IN YOUNG CHILDREN*

*By*

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Received for Publication—March 8, 1930

IN THE nutrition of a child thoroughness of utilization is as important as the ingestion of the proper food. Though many studies have been concerned with the fate of other foods, relatively few have considered the ability of the human organism to absorb and retain vitamin. The retention and storage of vitamin A is essential to the health of a child; therefore any study which throws light on its utilization should be of value. With other foods defective utilization can be traced to excretion from the alimentary tract or to loss through the urine. Urinary loss in the normal individual may indicate an excessive ingestion with an absorption so rapid that it overwhelms the metabolic processes of the organism. Cooper (1923) showed that excessive amounts of vitamin A in the diet of an adult were accompanied by excessive loss through the urine and believes that alimentary vitaminuria indicates a limited capacity of the organism to store or destroy vitamin A. Fecal loss may be caused by food being in a form difficult of digestion, by a too rapid passage through the intestine, or by excretion from the blood into the alimentary tract.

That utilization of food stuffs is dependent on the cellular structure of the food was shown by Rubner (1879). With a coarse bread or vegetable diet, the weight of the dried feces and the nitrogen loss were far greater than when a meat diet was fed. Sherman and Hawley (1922) found the calcium retention markedly less in children when a part of the calcium of milk was replaced by that of carrots and spinach, though Rose (1920) and Blatherwick and Long (1922) showed that vegetable calcium was well utilized by adults. Though adults may be able to digest such material, the feces of children are frequently found to contain large amounts of carrots, spinach or orange which have been ingested for their vitamin and mineral content. The presence of undigested foods in the feces of children makes it probable that both minerals and vitamins are lost through incomplete digestion. That impaired fat absorption also resulted in a loss of vitamin A was shown by Bloch (1924) who noted xerophthalmia in children

* Submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy in Nutrition, Department of Child Welfare, State University of Iowa, 1929.

with jaundice even when the vitamin A in the diet was adequate. Daniels (1925–26) found that a bottle-fed baby excreted more vitamin A through the feces than one who was breast fed. This may have been due to the bottle fed infant excreting more calcium soaps than one which is breast fed.

From the above it seems probable that poor retention of vitamin A may be caused by food being in an unavailable form, by an excessive amount in the diet, or by incomplete fat absorption. This study was undertaken to determine if, when a liberal amount of vitamin A is included in the diet of children, it is normally excreted in the urine; if there is significant loss through the feces; and if such loss is affected by changes in the diet.

*Subjects.* In the first experiment, which served primarily for orientation, eight children, four of whom were diabetic and four in normal health, served as subjects. The diet of the diabetic children contained from one hundred sixteen to one hundred and fifty grams of fat while the normal diets contained but sixty grams. The protein foods given were similar, all of the children being given one gram of protein per pound of body weight. The same vegetables prepared in the same way were served both groups, each child receiving approximately one hundred and fifty grams of vegetable other than potatoes.

In the main experiments four infants from two to eight months of age served as subjects. They were normal and healthy, being in Children's Hospital only for the purpose of metabolic research. Their basal formula was sweetened whole milk soured by addition of lactic acid. Cod liver oil and orange juice were administered in addition. Variations were made in the diet in order to note the effect on the excretion of vitamin A. At different times sieved carrots or the yolk of an egg were added, cod liver oil omitted and, during one period, skim milk was substituted for whole milk.

*Preliminary Trial with Color Test.* The problem was undertaken with the intention of using the Carr and Price (1926) color test to determine the amount of vitamin A in the food and secretions. Antimony trichloride in chloroform solution was said to combine with vitamin A and develop a deep blue color which could be measured with standard glasses in a tintometer. The test was tried on many solutions known to contain the vitamin. It could be used with cod liver oil, but with butter and egg yolk it proved most unsatisfactory. No color developed as long as a trace of water was present, nor would either food respond to the test after ordinary methods of freeing from water had been used. After this preliminary work was completed, the reports of Hawk (1929) and Willimott

and Wokes (1928) appeared, indicating that this test is not specific for vitamin A. The failure and *nonspecificity* of this test was unfortunate as chemical analysis would have been more accurate and far less subject to uncontrollable variations than assay by animal experimentation.

*Biological Method of Analysis Used.* Young rats were depleted of their store of vitamin A as shown by cessation of growth and xerophthalmia, and at this point the material to be tested was added; the relative content of vitamin A was determined by comparing the gains made by the rats. Young rats from our own colony, twenty-one to twenty-three days old, weighing from thirty-five to forty grams, were depleted of their store of vitamin A by feeding the following diet; casein eighteen per cent, crisco twenty per cent, salt mixture four per cent, compressed yeast twelve per cent, and cornstarch forty-six per cent. The casein was purified by continuous extraction with ninety per cent alcohol for a period of sixteen hours in a Clarke extractor as modified and described by Sperry (1926). The extraction was followed by heating for a week in an oven at 100° to 110°C. Previous to the extraction hot alcohol was poured over the casein and this alcohol drained off in twenty-four hours. This previous treatment prevents tunneling and caking of the casein in the extractor.

Vitamin D was given as irradiated ergosterol* and was diluted with olive oil and fed so that each rat received 2.5 milligrams of the activated material every other day, which would be approximately equivalent to 0.0002 milligrams of ergosterol. From the time of weaning the rats were kept in individual cages with elevated screen bottoms and were weighed twice weekly until ophthalmia appeared and after that on alternate days. No addition of material to be tested was made until the symptoms of ophthalmia had been evident for three successive days, at which time the weight was stationary or declining. When additions were made they were thoroughly incorporated in the basal diet and enough given at a time to last four days. In order to eliminate variations between litters, the rats were selected so that litter mates were distributed between the various extracts fed instead of using one litter for testing the excretion of one child.

*Preparation of Extracts.* This study involved the removal by fat solvents of the vitamin A from the excretions of children, and the feeding of the extracts to the rats. In order to determine the percentage of the ingested vitamin A excreted, the diets of the children were extracted in the same manner and fed to another group of rats. This procedure also served as a check on the method.

* Viosterol (Acterol) supplied by the Mead Johnson Co.

Twenty-four hour specimens of the feces and urine of the children were collected. The urine was evaporated almost to dryness on the water bath and was then extracted, first with hot alcohol and then with ether. The amount of each used for the extraction was eight per cent of the original amount of urine. The extract was then evaporated almost to dryness and the final residue suspended in alcohol and distilled water. It was impossible to collect the urine of the infants regularly. A collection was made for short periods, however, when they were on a metabolism frame, and a composite sample of three days extracted in the same way as the older children's.

An effective device was employed for collecting the infants' feces. Cotton wadding of the desired size was attached to heavy paraffin paper by means of a hot iron. This served to prevent any loss of fecal material by absorption into other garments. The wadding and feces were easily removed from the paraffin paper and transferred to extraction bags. The wadding served to prevent packing of the feces during the extraction. The feces were placed in pongee bags and hung under a small condenser in a flask and extracted continuously, first with the vapor from boiling alcohol and then in the same manner with ether. After three hours of extraction, the alcohol was removed and replaced by ether and the ether extraction continued for ten hours. The previous extraction with alcohol has two advantages. According to Bloor (1929) it penetrates tissues readily and breaks up loose combinations between lipids and cellular constituents and, owing to its affinity for water, dries the material, thus making a cleaner extraction than ether alone. The alcohol may also prevent oxidation of the vitamin during the drying. At intervals during the extraction the bags were pressed and the contents thoroughly mixed. The alcohol and ether extracts were combined, evaporated almost to dryness, then mixed with alcohol and water and fed in this form. The extractions were made daily, but the total extract from the feces of one child was combined before feeding so that the test was made not of one day's excretion, but of several.

The same apparatus was used with the feces of the children when the amounts were small. With larger quantities a large funnel, holding a wide-mouthed bag, served as a percolator. The alcohol which was kept boiling in the flask under the funnel was condensed by means of water flowing through a large, round-bottom flask which fitted tightly into the top of the funnel. The bag containing the feces was held away from the sides of the funnel by means of glass tubing bent into V shapes. This

allowed the alcohol to circulate freely. With the larger amounts of feces, a longer time was necessary for extraction, the process being carried on until, upon drying, the residue crumbled to a powder. There was no certainty that all of the vitamin A was removed by this process, but petroleum ether extracts of the residue showed only the slightest trace of fat on evaporation.

In order to test the adequacy of the method, however, the diets of the infants and children were treated in the same manner, and extracts prepared in the same way were fed control rats. These rats served not only as a check on the method, but also as a means of determining the approximate amount of vitamin A in the diet of the children in order that the amount ingested could be compared with the loss through the feces. For this purpose varying amounts of the extract of the diet were fed these rats, and their growth compared with that of litter mates which were receiving different amounts of the fecal extracts of the children.

*Basis for Amounts of Extract Fed the Rats.* In order to determine the amount of the extracts to be fed to each rat daily, the relative amounts of vitamin A in the food of the children and infants were calculated, using the tables compiled by Munsell and Burtis and reported by Sherman (1926). The calculation showed that the diabetic diet contained approximately 8,000 units, the diet of the normal children 6,000 and the food of the infants 2,000 to 4,000 units in the different periods. This unit is the amount of food which will produce a gain of three grams weekly in a rat and Sherman advises that this be used as a standard of comparison in assaying foods for vitamin A. Coward (1927), Luce (1924) and Morgan and Smith (1928) suggest that a greater gain be taken as a unit since there are fewer rats sacrificed and the rats maintained on the lower level are rendered abnormal because of their extremely low vitamin A margin. For this reason, all of the extracts were fed with the intention of producing the larger gain.

Though the general procedure in all the experiments was the same, on account of the type of extract and the age of the subject, the findings bear little relation to each other. For that reason the results of each will be given and discussed separately.

## Experiment I

### Growth of Xerophthalmic Rats on Extracts of Urine of Children

*Procedure.* After xerophthalmia had developed, a group of sixteen rats chosen from ten different litters was fed the extracts of urine from four

infants and four children all receiving generous amounts of vitamin A. In terms of the original urine, the amounts given the rats varied from twenty to five parts of urine to one part of food. When the larger amounts of the children's urine were fed, these amounts represented approximately one-half of their daily excretion, while the smaller amount of the infant's urine equaled one-tenth of the daily excretion.

TABLE I

RESULTS OF FEEDING XEROPHTHALMIC RATS VARYING QUANTITIES OF THE URINE EXTRACTS OF CHILDREN AND INFANTS WHO WERE RECEIVING DIETS HIGH IN VITAMIN A

| Rat | Sex | Days on A-free diet | Weight when depleted gms. | Urine Extract Fed | | | Days given | Loss gms. |
|---|---|---|---|---|---|---|---|---|
| | | | | Child | Diet of child | Ratio of urine to Food* gms. | | |
| 22A | M | 64 | 64 | B.M. | Normal | 10:1 | 8 | 6** |
| 2C | F | 50 | 102 | B.M. | " | 10:1 | 17 | 34** |
| 3C | F | 34 | 90 | A.K. | " | 10:1 | 14 | 15** |
| 17D | M | 26 | 80 | A.K. | " | 10:1 | 30 | 5** |
| 7E | M | 36 | 70 | A.K. | " | 20:1 | 13 | 12** |
| 29A | F | 33 | 70 | M.M. | " | 20:1 | 11 | 10** |
| 35B | M | 32 | 100 | R.P. | Diabetic | 10:1 | 7 | 32** |
| 176H | M | 49 | 146 | T.S. | Infant | 5:1 | 18 | 46** |
| 205A | M | 36 | 80 | M.P. | " | 5:1 | 20 | 4** |
| 242H | F | 30 | 80 | M.P. | " | 5:1 | 14 | 0** |
| 210B | M | 38 | 84 | T.S. | " | 5:1 | 22 | 18** |
| 278L | F | 32 | 84 | T.S. | " | 5:1 | 17 | 14** |
| 235G | M | 45 | 135 | T.S. | " | 5:1 | 16 | 23** |
| 4C | F | 34 | 98 | A.K. | Normal | 10:1 | 17 | 20† |
| 24A | F | 42 | 84 | R.P. | Diabetic | 10:1 | 8 | 10† |
| 202A | F | 45 | 96 | R.T. | Infant | 5:1 | 28 | 16† |

* In terms of original urine.

** Died.

† Cod liver oil given at this point.

The diet of the infants at this time consisted of approximately one quart of whole milk, one egg yolk, one teaspoon of cod liver oil, and sixty grams of carrots daily. Two of the children were diabetic (R.P. and D.H.) and were receiving two eggs, one teaspoon cod liver oil, and 150 cc. of forty per cent cream. The other two children were getting a normal diet containing approximately three-fourths of the amount of vitamin A given the diabetic children.

*Results.* All of the depleted rats failed to show even slight improvement,

and there was little difference in the survival period of those given the smaller and larger amounts of urine extract. Table I shows that the rats fed the urine of the diabetic children, who were receiving large amounts of vitamin A in their diet, survived no longer than those fed the extracts from the children on the normal hospital diet. Therefore it appears that the rats received no vitamin A from the urine of these children. They not only lost weight markedly, but became blind before they died and in as many as ten cases the eyeballs were ruptured. Thirteen died within a month of the development of xerophthalmia, and the other three were in an almost moribund condition when cod liver oil was added to their ration to note if improvement would take place. Although it seemed very evident that death was due to a lack of vitamin A, there was a possibility that the alcohol and ether might have extracted substances from the urine which were toxic to the rats. For this reason, when it was evident that three of the rats (numbers 4, 24, and 202) would not survive on the urine extracts given, one drop of cod liver oil was added daily to the urine extract ration to determine whether their pitiable condition was due to a lack of vitamin A or to a toxic material extracted from the urine. The improvement was immediate. During the next two weeks the average gain was thirty grams, and it was evident that their previous loss was due to a lack of vitamin A. The results are shown in Table I.

Since administration of vitamin A produced marked improvement in these rats and one-tenth to one-half of the daily urinary excretion of a child failed to produce any improvement, it seems certain that no vitamin was lost through the urine of these infants and children.

*Discussion.* These results were surprising, inasmuch as they were so different from the findings of Cooper (1924). She found that adults on high vitamin A diets excreted urine which contained sufficient vitamin A to produce marked gains in a rat when only five parts of urine to one part of food was fed. The children in this experiment were receiving generous though not excessive quantities of vitamin A, but our rats were fed twice the amount of urine extract given by Cooper. Since she was able to deplete in only two weeks rats which were six weeks old when put on a vitamin A-free diet, one is led to wonder if her diet was adequate in other respects. Steenbock, Sell, and Nelson (1923) and Sherman and coworkers (1925, 1926, 1928) have shown that it requires five to six weeks to deplete a rat of the vitamin A stored in its tissues even if put on the vitamin A-free diet when three or four weeks old. The longer it is given an adequate diet after weaning, the longer the time necessary for the de-

pletion of these stores. Cooper (1924) gave an adequate diet for two weeks after weaning, and states that after two weeks on the vitamin A-free diet the rats ceased to gain, or actually lost weight. This early loss of weight appears more like a vitamin B deficiency. Since Cooper did not use yeast as a source of vitamin B but used an alcohol extract of yeast, and since its potency was determined on polyneuritic pigeons only, it may have lacked the P-P heat-stable factor. Sherman and Sandels (1928) have recently shown that the P-P fraction of vitamin B is not soluble in eighty or ninety-five per cent alcohol. It seems probable, therefore, that the growth of Cooper's rats was resumed because urine extract supplied not vitamin A but vitamin G, the heat-stable fraction of vitamin B. Muckenfuss (1918) showed that human urine contains vitamin B. Though there is a possibility that the vitamin A may have been oxidized during our drying process, the same method was employed as that used by Cooper (1923) with the extra precaution of the addition of alcohol to prevent oxidation. It appears conclusive, therefore, that no vitamin A was excreted in the urine of these children whose diets contained generous amounts of the vitamin.

## Experiment II

### Growth of Xerophthalmic Rats on the Fecal Extracts of Pre-School Children

*Procedure.* Fifteen rats were fed the extracts of fecal matter of eight different children, four of whom were diabetic and four normal. Both the groups were receiving liberal amounts of vitamin A, the diabetic approximately one-third more than the normal children. As there had been no previous work done to show the amount of vitamin A lost through the feces, six per cent of the extract of the daily excretion of the children was chosen as a reasonable amount to test. Two of the rats were fed but half this amount. As controls, rats from the same litters were fed varying amounts of similarly prepared extracts of the diet of the normal children. The amounts fed were 0.05, 0.15, and 0.45 per cent of the food extract.

*Results. Fecal Excretion of Vitamin A.* The growth of the thirteen rats (see Table II) showed that six per cent of the fecal extract was adequate to produce normal gains, since during the next twenty-five days an average daily gain of three grams was made. Those fed the extracts of the diabetic feces made no greater gains than those fed the extracts of the feces of normal children. A gain of three grams daily was in all probability maximum for the rats, so that this similarity of gain would not necessarily

show that all rats were receiving the same amount of vitamin A. The rate of gain of those getting six per cent of the fecal extract was almost identical with that of those receiving 0.45 per cent of the food extract (see Table IV). Since the amount of vitamin A in these two extracts is approximately the same, it would appear that the children were losing seven and a half per cent of the ingested vitamin A through the feces.

$$0.45\%:6\%::\times:100$$
$$\times=7.5$$

TABLE II

GROWTH OF RATS FED SIX AND THREE PER CENT OF THE DAILY FECAL EXTRACTS OF NORMAL AND DIABETIC CHILDREN OF PRE-SCHOOL AGE

| Rat | Sex | Days on A-free diet | Weight when depleted gms. | Fecal Extracts Tested | | | Gain gms. | Diet of child |
|---|---|---|---|---|---|---|---|---|
| | | | | Child | Amt. given Per cent | Days given | | |
| 1C | F | 49 | 106 | D.H. | 6 | 25 | 37 | Diabetic |
| 23A | F | 35 | 80 | D.H. | 6 | 25 | 68 | " |
| 18H | M | 31 | 74 | R.P. | 6 | 25 | 66 | " |
| 11E | M | 46 | 106 | D.M. | 6 | 25 | 70 | " |
| 27A | F | 36 | 74 | E.B. | 6 | 25 | 75 | " |
| 28A | M | 36 | 72 | E.B. | 6 | 25 | 72 | " |
| 68G | M | 35 | 90 | W.A. | 3 | 25 | 46 | " |
| 69G | M | 35 | 122 | W.A. | 3 | 25 | 64 | " |
| 36B | F | 37 | 102 | B.H. | 6 | 25 | 48 | Normal |
| 19H | M | 43 | 88 | B.M. | 6 | 25 | 32 | " |
| 6A | F | 38 | 88 | A.K. | 6 | 25 | 48 | " |
| 14D | M | 42 | 74 | A.K. | 6 | 25 | 100 | " |
| 16D | M | 37 | 74 | A.K. | 6 | 25 | 90 | " |
| 25A | F | 32 | 68 | M.M. | 6 | 25 | 74 | " |

This alone shows little since both groups of rats were doubtless receiving enough vitamin A to make the maximum growth; but the two fed half as much fecal extract, three per cent, and half the amount of food extract, also made almost equivalent gains so these figures would still indicate that between seven and eight per cent of that ingested was lost through the feces.

It is possible that all of the rats would have made normal gains on a smaller amount of fecal extract than they received. But the experiment showed that the children were excreting vitamin A through the feces and that the methods of extraction were satisfactory. Since it was impossible to vary the diet of these children markedly, there was no way of determin-

ing whether the fecal loss was the result of the type of food fed. Since this was the primary purpose, the second part of the study was carried on with infants who would normally have their diets gradually changed. But before proceeding it seemed wise to make certain that the growth made by the rats was due to vitamin A alone and not to other substances extracted from the feces by alcohol and ether.

*Validity of Experiments.* In order to make certain that the growth of the rats was due to the vitamin A and not to other substances extracted from the feces, the vitamin A present in the extract of fecal matter of one child was oxidized by means of ferrous sulfate and exposure to air. The rate of gain of rats fed this extract was compared with that of others fed the extract in its unoxidized form. Jones (1927), and Simmonds, Becker, and McCollum (1927), have shown that as little as 0.02 per cent of the ration in ferrous sulfate is sufficient, but to make certain that oxidation would take place 0.06 per cent ferrous sulfate was added. This quantity was incorporated in the extract, added to the food, and the mixture spread out and allowed to stand in a warm place for a month before feeding. This mixture and another portion of the same extract, unoxidized, were fed to groups of four rats each.

The four rats fed the fecal extract in which the vitamin A had been oxidized gradually lost weight, their average period of survival being twenty-four days. The four fed the unoxidized extract made an average gain of forty-seven grams during the twenty-five day test period. The fact that those fed the oxidized extract survived as long showed that there was no toxic effect from the alterations and additions in each period.

Collections were made on two infants for seven periods, on one for six periods, and on one for only three periods. The last two infants were not very robust and it seemed unwise to leave cod liver oil out of the diet of the one or to feed either child skim milk alone. Each diet was fed for nine days at least, and fecal collections were made during the last six days. Very often the fore period was longer than three days, but this length of time served to accustom the child to the diet and to eliminate from his alimentary tract the residues from other diets. These infants were in the metabolism ward and under the constant supervision of special nurses. The whole of the prescribed diets was given, even though at times the process was laborious. The feces were extracted daily, but the extracts of the six days were combined before feeding to the rats, so that analysis of the feces for vitamin A was made not on one day's excretion but on the combined excretion of six days.

In order to have a basis of comparison between the amount of vitamin ingested and that excreted, the diets were assayed as in the previous experiment by feeding extracts of them in varying amounts to litter mates of the rats fed the fecal extracts, and by comparing the rates of growth pro-

TABLE III

FOOD, WEIGHT, AND AGE OF INFANTS DURING DIFFERENT PERIODS OF FECAL COLLECTIONS

| Child..... | | M.R. | | H.D. | | R.B. | | C.P. | |
|---|---|---|---|---|---|---|---|---|---|
| Period | Food* | Age wks. | Weight kilos. | Age wks. | Weight kilos. | Age wks. | Weight kilos. | Age wks. | Weight kilos. |
| I | Basal, 55 cc. corn sirup, 1 oz. orange juice, 1 tsp. cod liver oil | 18 | 8.8 | 9 | 5.3 | 13 | 6.4 | 13 | 5.8 |
| II | Basal without cod liver oil | 20 | 9.2 | 15 | 6.8 | 17 | 8.1 | | |
| III | Basal with egg yolk, no cod liver oil | 22 | 9.6 | 13 | 6.1 | 18 | 8.3 | | |
| IV | Basal with carrots | 30 | 9.8 | 17 | 7.2 | 20 | 8.5 | | |
| V | Basal with egg yolk and cod liver oil | 34 | 10.0 | 18 | 7.4 | 23 | 7.2 | 23 | 8.2 |
| VI | Basal with egg yolk, carrots, and cod liver oil | 31 | 9.9 | 22 | 7.5 | 26 | 9.0 | 26 | 9.0 |
| VII | Skim Milk in place of whole; no additions | 32 | 10.2 | 21 | 7.8 | | | | |

* Amount of milk given each infant was not changed during the seven periods. Amounts given each were: M.R., 900 cc.; H.C., 810 cc.; R.B., 870 cc.; C.P., 800 cc. One teaspoon of cod liver oil, the yolk of one egg, and sixty grams sieved carrots were the amounts given in each case.

duced by different percentages of the two extracts. The approximate amount contained in each diet was previously calculated in units from the tables given by Sherman (1926) in order to estimate how much of each food extract to feed the rats. The gains in the rats showed that on the whole the vitamin A content of these diets was lower than the amount estimated.

From the calculations it appeared that the basal diet contained about 2,000 units; the basal with cod liver oil approximately 3,000; the basal with egg also about 3,000; basal with egg yolk and cod liver oil 4,000; basal with carrots nearly 3,000; and the complete diet, containing all additions, nearly 5,000. Sherman lists no vitamin A values for skim milk,

TABLE IV

GAINS IN WEIGHT OF RATS FED VARYING AMOUNTS OF EXTRACTS OF DIETS OF INFANTS, SHOWING EFFECT OF CHANGES IN DIET OF THE CHILD

| Period | Rat | Sex | Days on A-free diet | Weight when depleted gms. | Extract of Diet | | Loss or gain gms. |
|---|---|---|---|---|---|---|---|
| | | | | | Amt. daily Per cent | Days given | |
| I | 60F | M | 40 | 130 | 0.33 | 25 | 40 |
| Basal with cod | 63F | F | 43 | 116 | 0.33 | 25 | 30 |
| liver oil | 64F | M | 43 | 120 | 0.33 | 25 | 30 |
| | 97M | M | 31 | 130 | 0.16 | 25 | 24 |
| | 295O | M | 45 | 102 | 0.16 | 25 | 16 |
| III | 126X | M | 47 | 142 | 0.03 | 25 | −18* |
| Basal with egg | 160O | F | 44 | 134 | 0.12 | 25 | 16 |
| yolk | 168B | M | 35 | 116 | 0.12 | 25 | 0 |
| | 212B | M | 18 | 55 | 0.12 | 25 | 20 |
| | 249H | F | 30 | 50 | 0.12 | 25 | 16 |
| VI | 238G | M | 45 | 132 | 0.12 | 25 | 50 |
| Basal plus all | 244H | F | 42 | 105 | 0.12 | 15 | 42 |
| other ingredi- | 252I | M | 38 | 88 | 0.12 | 25 | 50 |
| ents | 114Y | F | 38 | 150 | 0.09 | 25 | 34 |
| | 228F | F | 42 | 124 | 0.06 | | 30 |
| VII | 158O | M | 34 | 120 | 1.00 | 25 | 0 |
| Skim milk | 255II | M | 38 | 128 | 0.5 | 20 | −6 |
| | 269K | M | 38 | 135 | 1.00 | 26 | −28 |

* Died

but Hart (1925) believes that skimming removes ninety per cent of the vitamin, so this diet, during Period VII, under these conditions would not contain more than 200 units.

*Results. Growth Induced by Extracts of Diets.* When the extracts of these diets were fed the rats, it will be seen in Table IV that 0.33 per cent of the diet during Period I (basal plus cod liver oil) produced an average gain of but ten grams weekly when fed three rats, and that half this

amount produced a gain of but four grams. Assuming that this diet contained 3,000 units, then ten units were required to produce a gain of ten grams weekly. The three rats fed the diet in which egg yolk was substituted for cod liver oil gained five grams weekly when 0.12 per cent of the diet (3.6 units) was supplied daily. The rats fed 0.12 per cent of the complete diet, Period VI, gained fifteen grams weekly on an intake of six units daily. It appears, therefore, that the alcohol-ether extraction of the diet removed the vitamin A from the diet very efficiently. Though the gains in the rats were somewhat lower than would be expected, this could easily be explained by the fact that winter milk was used during the entire experiment. When the extract of the skim milk diet was fed, it was found that almost all of the vitamin A was removed by the skimming process, as one per cent of the diet produced no gain in any of the rats. From the analysis, therefore, it can be concluded that the diet during Period VII was devoid of vitamin; that the diets during Periods I, III, and V were about half as rich in vitamin as during Periods IV and VI, and twice as rich as in Period II.

*Growth Induced by Extracts of Feces.* When the diets of the infants were thus varied, the feces were also quite different in vitamin content. The results are given in Tables V to VIII. They are arranged so that the vitamin A excretion of each child can be compared with that of his own succeeding or previous period. When the fecal extracts during the different periods were found to vary so markedly, it did not seem feasible to give exactly the same percentages of each to the different rats. Inasmuch as one per cent was sufficient for normal growth in one case and in another quite inadequate, the quantities of fecal extract fed varied from six to one per cent during the different periods.

Because the amount of vitamin A given off by the children was so much greater than was expected, the percentages of the fecal extracts fed the rats were seldom small enough to produce the intended subnormal growth. It was assumed that one per cent of an infant's feces would not contain enough vitamin to make the rats gain more than ten to twelve grams weekly. Such, however, was not the case. During periods of highest vitamin A intake, one per cent of the feces induced not a subnormal but a maximum gain; while during periods when the vitamin A intake of the children was low, the same percentage of this fecal extract was too small even for maintenance. For this reason, the data are difficult of comparison. Since a definite percentage of the different extracts cannot be used as a basis, Table IX has been arranged to show what percentage of the food

TABLE V

GAINS IN WEIGHT OF RATS FED VARYING QUANTITIES OF FECAL EXTRACTS OF INFANT M.R., SHOWING EFFECT OF CHANGES IN DIET OF CHILD

| Period | Rat† | Sex | Days on A-free diet | Weight when depleted gms. | Fecal Extract Fed | | Gain or loss gms. |
|---|---|---|---|---|---|---|---|
| | | | | | Amt. daily Per cent | Days given | |
| I | 56H | M | 38 | 132 | 6 | 25 | 64 |
| Basal with cod | 48J | M | 35 | 106 | 6 | 25 | 65 |
| liver oil | 52H | F | 38 | 135 | 6 | 25 | 64 |
| | 76K | M | 35 | 130 | 3 | 25 | 40 |
| II | 87L | F | 30 | 114 | 4 | 10 | 0* |
| Basal only | 119Y | M | 56 | 148 | 3 | 19 | −32 |
| | 115U | M | 49 | 125 | 3 | 7 | −40* |
| | 61F | F | 39 | 135 | 3 | 11 | −30* |
| | 62F | F | 39 | 100 | 3 | 10 | −20* |
| | 96M | M | 25 | 100 | 3 | 10 | −20* |
| | 200A | F | 45 | 99 | 2 | 19 | −20* |
| III | 111Y | F | 23 | 98 | 4 | 25 | 10 |
| Basal plus egg | 110Y | M | 38 | 115 | 3 | 25 | 4 |
| yolk | 175H | M | 33 | 120 | 2 | 25 | 5 |
| | 226E | F | 37 | 98 | 1 | 20 | −20* |
| IV | 124X | M | 46 | 164 | 4 | 25 | 48 |
| Basal plus egg | 121X | F | 44 | 142 | 3 | 25 | 40 |
| and cod liver oil | 125F | F | 48 | 150 | 3 | 25 | 34 |
| | 217M | M | 38 | 110 | 1 | 25 | 20 |
| V | 140R | F | 56 | 160 | 3 | 25 | 15 |
| Basal plus car- | 142R | F | 56 | 165 | 3 | 25 | 9 |
| rot | 149S | M | 49 | 130 | 3 | 25 | 20 |
| | 174H | M | 38 | 138 | 2 | 25 | 20 |
| | 177H | M | 53 | 116 | 2 | 25 | 12 |
| | 233F | F | 47 | 130 | 2 | 25 | 14 |
| VI | 154Q | M | 48 | 140 | 3 | 25 | 55 |
| Basal plus all | 172H | F | 33 | 122 | 2 | 25 | 36 |
| other ingredi- | 173H | F | 33 | 108 | 2 | 25 | 46 |
| ents | 156Q | F | 47 | 140 | 1 | 25 | 18 |
| | 267K | M | 36 | 116 | 1 | 25 | 16 |
| | 282M | F | 47 | 130 | 0.5 | 25 | −30* |
| VII | 139R | F | 55 | 150 | 3 | 25 | 18 |
| Skim milk | 161O | M | 41 | 154 | 3 | 25 | 30 |
| | 170H | M | 40 | 108 | 2 | 25 | 20 |
| | 169H | M | 40 | 138 | 2 | 25 | 12 |
| | 231F | F | 43 | 132 | 1 | 25 | 25 |
| | 294O | F | 45 | 108 | 1 | 25 | −8 |

* Died.

† Similar letters indicate litter mates.

TABLE VI

GAINS IN WEIGHT OF RATS FED VARYING AMOUNTS OF FECAL EXTRACTS OF INFANT H.D., SHOWING EFFECT OF CHANGES IN DIET OF THE CHILD

| Period | Rat | Sex | Days on A-free diet | Weight when depleted gms. | Fecal Extract Fed | | Gain or loss gms. |
|---|---|---|---|---|---|---|---|
| | | | | | Amt. daily Per cent | Days given | |
| I | 20A | F | 35 | 84 | 3 | 25 | 39 |
| Basal plus cod | 71G | M | 29 | 105 | 6 | 25 | 60 |
| liver oil | 51H | F | 29 | 115 | 6 | 25 | 65 |
| | 53H | F | 35 | 85 | 6 | 25 | 46 |
| | 41B | F | 33 | 95 | 6 | 25 | 65 |
| | 40B | F | 45 | 90 | 3 | 25 | 35 |
| | 72G | M | 27 | 112 | 3 | 25 | 68 |
| | 70G | M | 29 | 110 | 3 | 25 | 75 |
| | 49J | F | 32 | 102 | 6 | 25 | 60 |
| II | 145S | F | 56 | 150 | 3 | 25 | 10 |
| Basal | 289N | F | 34 | 98 | 3 | 25 | 30 |
| | 209B | M | 34 | 88 | 2 | 25 | 4 |
| | 218C | M | 45 | 118 | 2 | 25 | 12 |
| III | 66F | M | 45 | 122 | 6 | 25 | 58 |
| Basal plus egg | 65F | F | 45 | 160 | 6 | 25 | 60 |
| yolk | 113Y | F | 37 | 128 | 3 | 25 | 30 |
| IV | 243H | F | 34 | 88 | 2 | 25 | 52 |
| Basal plus cod | 258J | F | 40 | 96 | 2 | 25 | 54 |
| liver oil and egg | 263J | M | 39 | 120 | 1 | 25 | 60 |
| yolk | 245H | F | 34 | 75 | 1 | 25 | 60 |
| V | 219C | M | 49 | 128 | 2 | 25 | 68 |
| Basal plus car- | 261J | F | 39 | 112 | 1 | 25 | 34 |
| rot | 260J | M | 39 | 102 | 1 | 25 | 38 |
| VI | 211B | F | 38 | 90 | 2 | 25 | 68 |
| Basal plus all | 216C | M | 37 | 94 | 1 | 25 | 60 |
| other ingredi- | 248A | M | 33 | 68 | 1 | 25 | 32 |
| ents | 273K | F | 32 | 100 | 1 | 25 | 70 |
| | 298O | F | 20 | 88 | 1 | 25 | 48 |
| VII | 167P | M | 47 | 128 | 3 | 25 | 30 |
| Skim milk | 207A | F | 42 | 80 | 2 | 25 | 30 |
| | 297O | M | 42 | 112 | 2 | 25 | 20 |
| | 204A | F | 42 | 90 | 2 | 25 | 0 |
| | 270K | M | 33 | 72 | 1 | 25 | Died |

TABLE VII

GAINS IN WEIGHT OF RATS FED VARYING AMOUNTS OF FECAL EXTRACTS OF INFANT R.R., SHOWING EFFECT OF CHANGES IN DIET OF THE CHILD

| Period | Rat† | Sex | Days on A-free diet | Weight when depleted gms. | Fecal Extract Fed | | Gain or loss gms. |
|---|---|---|---|---|---|---|---|
| | | | | | Amt. daily Per cent | Days given | |
| I | 47J | F | 44 | 128 | 6 | 25 | 50 |
| Basal plus cod | 50J | ** | 31 | 122 | 3 | 25 | 26 |
| liver oil | 76K | M | 37 | 140 | 3 | 25 | 20 |
| | 213B | M | 49 | 142 | 2 | 25 | 20 |
| | 74K | M | 30 | 84 | 2 | 12 | −10* |
| II | 112Y | M | 44 | 140 | 6 | 25 | 24 |
| Basal alone | 150S | F | 56 | 128 | 6 | 25 | 24 |
| | 151S | M | 56 | 136 | 3 | 25 | 12 |
| | 224E | F | 46 | 102 | 3 | 25 | 8 |
| | 146S | F | 56 | 160 | 2 | 25 | −12 |
| | 224E | F | 47 | 82 | 1 | 16 | −14* |
| III | 99M | F | 31 | 100 | 6 | 25 | 54 |
| Basal plus egg | 89L | F | 40 | 110 | 1 | 25 | 52 |
| yolk | 84L | M | 41 | 92 | 6 | 25 | 44 |
| | 98M | F | 35 | 85 | 4 | 25 | 50 |
| | 82L | M | 36 | 108 | 3 | 25 | 42 |
| | 94M | M | 39 | 120 | 3 | 25 | 36 |
| IV | 277L | M | 38 | 110 | 2 | 25 | 88 |
| Basal plus cod | 276L | M | 32 | 88 | 1 | 25 | 88 |
| liver oil and egg | 217C | M | 38 | 110 | 1 | 25 | 60 |
| yolk | 288N | M | 34 | 98 | 1 | 25 | 68 |
| V | 135W | F | 53 | 150 | 3 | 25 | 28 |
| Basal plus car- | 153Q | M | 55 | 150 | 3 | 25 | 40 |
| rots | 133U | F | 53 | 124 | 3 | 25 | 42 |
| | 215C | ** | 44 | 110 | 3 | 25 | 70 |
| | 272K | M | 36 | 90 | 1 | 25 | 30 |
| VI | 206A | M | 47 | 116 | 2 | 25 | 78 |
| Basal plus all | 208B | F | 45 | 90 | 2 | 25 | 70 |
| other ingredi- | 246H | M | 35 | 88 | 1 | 25 | 60 |
| ents | 247H | F | 36 | 100 | 1 | 25 | 53 |

* Died

† Similar letters indicate litter mates.

** Unrecorded

and excretions during the different periods would be required to produce the gain which Luce (1924) suggests should be used as a standard, ten to twelve grams weekly.

Since this table indicates the amount of extract required to produce a standard gain, the smallest figures indicate the greatest amount of vitamin. For example, when twelve per cent of the daily food or excretion

TABLE VIII

GAINS IN WEIGHT OF RATS FED VARYING AMOUNTS OF FECAL EXTRACTS OF INFANT C.P., SHOWING EFFECT OF CHANGES IN DIET OF THE CHILD

| Period | Rat | Sex | Days on A-free diet | Weight when depleted gms. | Fecal Extract Fed | | Gain gms. |
|---|---|---|---|---|---|---|---|
| | | | | | Amt. daily Per cent | Days given | |
| I | 44B | F | 50 | 110 | 6 | 25 | 34 |
| Basal plus cod | 50H | M | 34 | 150 | 6 | 25 | 60 |
| liver oil | 46B | M | 50 | 150 | 6 | 25 | 62 |
| | 43B | M | 43 | 112 | 3 | 25 | 53 |
| | 55H | F | 36 | 60 | 3 | 25 | 26* |
| IV | 280M | F | 33 | 110 | 1 | 25 | 10 |
| Basal plus cod liver oil and egg yolk | 230F | M | 33 | 110 | 1 | 25 | 12 |
| VI | 236G | F | 47 | 126 | 1 | 25 | 48 |
| Basal plus all other ingredients | 256I | M | 48 | 112 | 1 | 25 | 50 |

* Snuffles.

is required, the vitamin A content is much less than when only three per cent is needed. It is evident that with all four infants the greatest loss took place when the intake was highest, during Periods IV and VI. The amounts lost during Periods II and VII were smallest and those were also the periods of lowest vitamin intake. If a comparison is made of the required percentage of food and fecal extracts, it will be seen that during Period I, 0.33 per cent of the food extract produced the same gain as two and one-half to four per cent of the fecal extracts, indicating that eight to thirteen per cent of the vitamin ingested by the different children was excreted. Almost identical amounts were lost during Period III, but considerably greater losses during Period VI when more vitamin was given.

The results indicate that the youngest child (H.D.) lost much of the vitamin contained in the carrots, but the other two excreted no more than when given egg or cod liver oil. It is rather significant that the healthiest infant (M.R.) making the most rapid gains should have consistently lost

TABLE IX

COMPUTED PERCENTAGES OF THE EXTRACTS OF DAILY FOOD AND FECES OF FOUR INFANTS WHICH WOULD BE REQUIRED TO PRODUCE A GAIN OF TEN TO TWELVE GRAMS WEEKLY IN A RAT

| Period | Food | Food extracts Per cent | Fecal Extract of Infant | | | |
|---|---|---|---|---|---|---|
| | | | M.R. Per cent | R.B. Per cent | H.D. Per cent | C.P. Per cent |
| I | Basal plus cod liver oil | 0.33 | 3 | 4 | 2.5 | 3 |
| II | Basal | | 12 | 6 | 6 | |
| III | Basal plus egg yolk | .036 | 4 | 3 | 4 | |
| IV | Basal plus egg and cod liver oil | * | 3 | 0.6 | 0.6 | 1 |
| V | Basal plus carrots | * | 6 | 3 | 1.5 | |
| VI | Basal plus egg, cod liver oil and carrots | 0.12 | 2 | 0.75 | 0.75 | 0.75 |
| VII | Skim milk | Undet. | 5 | | 3 | |

* Diet not assayed.

the smallest amount of vitamin. The data indicate that he utilized both egg and carrots better than the other children. During the period of highest vitamin intake, his loss was only one-third as great as that of the other children. The amounts of vitamin A excreted by the other three infants are rather similar and coincide rather closely with the intake.

It is conclusively shown that, irrespective of the amount in the diet, all of the children were excreting some vitamin A through the feces. The two children receiving the skim milk during Period VII gave off a larger amount through the feces than they ingested.

The fact that the rats made a better gain on one per cent of the fecal extract than on one per cent of the extract of the skim milk diet itself, makes it evident that the excretion of vitamin A is not altogether dependent on the amount in the diet. However, in every case the losses were greatest during the period of high vitamin A intake and smallest during the periods when less was given. Throughout the experiment the larger intakes were accompanied by the larger outputs, but in no case was more than sixteen per cent of the dietary vitamin excreted, and thus in every case as much as eighty-four per cent was retained. Since eighty-four per cent of the diet high in vitamin A is a much larger amount than ninety-four per cent of the diet low in vitamin A, the results might be stated conversely—the higher the intake the greater the retention.

## Discussion

The fecal excretion of vitamin A by bottle-fed babies was not surprising in view of the work of Daniels (1925). That such excretion should take place with a diet low in vitamin A was unexpected. It would be of interest to discover whether complete retention is a characteristic of breast-fed babies or peculiar to the subject of Daniels' study. If a characteristic of breast-fed infants, it may be assumed that the loss of vitamin A takes place through the calcium soaps which the bottle-fed infant excretes in greater quantities than one who is breast fed. However, the fact that more vitamin A is found in the feces than in the food suggests that it is a component of the fat secretion of the intestine, and that re-absorption may be prevented by the formation of calcium soaps. Since these infants were fed the skim milk diet but nine days in all, with collections during the last six, it is quite possible that their secretions were richer in vitamin A than they would have been if maintained longer on the same diet. Probably the loss of vitamin A runs parallel to the loss of fat. It has been rather conclusively demonstrated that the fat in the feces is not the fat of the diet. Hill and Bloor (1922) as well as Holmes and Kerr (1924) have shown that fecal lipids differ from the fat ingested in both iodine number and saponification value. As much as 50 grams of fat in the diet increased fecal lipids only about fifty per cent over that on a fat-free diet. Nieman (1912) found that a child with an obstruction of the bile duct gave off in three days fifteen grams more fat than she ingested. He believes that fat is excreted from the blood into the intestine but re-absorbed to a large extent under normal conditions. Bloor (1929) found in an analysis of the five-day secretion of a fourteen-inch Thiry fistula that it contained 0.72

gram of lipid material. The work of Voit (1892) showed that an intestinal loop contained fat and fatty acid in greater quantity than is normally found in the feces, which may indicate that these substances are normally re-absorbed. Sperry's work (1926) also indicates that there is a secretion of lipids throughout the length of the intestine which either serves a useful purpose or represents leakage from the blood. Angevine (1929) has found little difference either in the total lipids or the type of lipids in the secretions of the intestinal loops of dogs when fed on different types of diet. The diets were either high, normal, or very low in fat content, but exerted no influence on the type of material secreted into the loop. It seems, therefore, that the secretion is rather constant in quantity and quality.

Sperry and Bloor (1924) have noted a close similarity between the plasma and fecal lipids. They suggest that this excretion of lipid material into the intestine may be due either to fat plethora and analagous to alimentary glycosuria, or to a true excretion of unusable waste fat from lipid metabolism. If the former assumption is correct, the fecal fat loss would depend upon the nutritive condition of the animal, the best nourished eliminating the largest amount. Talbot (1911) believed that the fecal fat loss of the infant represented incomplete fat digestion, but later results do not support his original opinions. It seems, therefore, that fecal fat represents in a large measure excretion from the blood into the intestine.

Whatever its source, there is fat loss in the feces, and vitamin A appears to be associated with it. Talbot found that the fecal fat loss in babies was from two to ten per cent of the fat ingested, and Holt, Courtney, and Fales (1919) found that infants receiving cow's milk excrete on an average eight and four-tenths per cent of the fat ingested when the stools are normal and ten per cent when constipated. The vitamin A loss was found to be rather similar to the fat loss. Whether the vitamin A loss is due to incomplete absorption of food or to excretion of vitamin A into the intestine is not of great practical importance since both forms represent wastage of vitamin consequent upon the digestion of food.

### Comparison of Vitamin A Content of Fecal Extracts of Infants on a Skim Milk Diet with that of Dogs on a Lipid-Free Diet

In view of the fact that children fed a short time on a diet very low in vitamin A lose a considerable amount of the vitamin in the feces, it seemed

probable that the dogs which Sperry (1926, 1927) fed on the lipid-free diet might also excrete vitamin A. He found that those dogs excrete from one to two grams of non-volatile lipids per week, and that this excretion continues for as long as five weeks when the dog is maintained on a lipid-free diet.

Dr. Sperry kindly furnished us with three portions of the non-saponifiable fraction of fecal lipids. Each portion represented an aliquot of the non-saponifiable fraction of three dogs for a week. In order to determine if any vitamin A was present, these fractions were fed to five rats previously depleted of their store of vitamin A. Each rat was given one-fourth of the non-saponifiable fraction of the excretion of a dog daily.

None of the rats showed any improvement. It cannot be concluded however that the dogs excreted no vitamin A, since it may have been destroyed during the separation of the lipids or oxidized by the ether in which it had been kept. Dogs kept so long a time on diets devoid of lipids may have excreted no vitamin A.

*Criticism of the Biological Method of Determining Content of Vitamin A.* Though definite conclusions can be drawn from the data given in the previous tables, there are certain fundamental weaknesses in the biological method of analysis which cannot be overcome. In this study a sufficient number of rats was used, over two hundred in all, to eliminate the danger of drawing conclusions from insufficient data. Enough controls were used to show that the basal diet was always free from vitamin A and in addition to these controls twenty-five other rats fed extracts totally lacking in vitamin A served the same purpose. The rats were kept in individual cages with screen bottoms throughout the experiment, were weighed and examined regularly for abnormalities and rejected if pneumonia became chronic before their eyes were affected. Xerophthalmia for three successive days was used as the criterion of depletion of vitamin A and no additions were made until it was thus established. As a rule it appeared at the time the weight became stationary, but in some cases the rats ceased to gain a week before the eyes were affected and the animals showed evidences of respiratory infections. If the rat was in a generally poor condition when the extract was added, it improved more slowly than those whose eyes only were affected.

Some dissimilarity was due to the size of the rats when their store of vitamin was depleted, the smaller rats making a quicker and more pronounced response than the larger. Members of the same litter would evidence xerophthalmia within a week of each other, but the difference in their

weights at the time would often be as great as thirty to forty grams. A further variation was the result of a change in the stock diet made necessary by the failure of the Sherman Stock Diet B to produce normal young. During the winter the condition of the stock females indicated nutritive failure; the young ceased to grow at the end of two weeks and many mothers killed their young. In order to save the remainder of the young for further work, boiled soy beans were added to the diet and liver given twice a week. The young were saved, but had to be rejected for this experiment because they had stored so much vitamin A that their growth was nearly complete before ophthalmia appeared. After this experience the Sherman Diet B was again employed but supplemented daily with fresh lettuce. Whatever the cause may have been, the result has again laid emphasis on the importance of the stock diet in the development of ophthalmia. The more adequate the stock diet, the greater is the difficulty in depletion.

## Conclusions

The results show that vitamin A is not excreted through the urine of young children and infants even when generous amounts are fed. If there is a limit to their capacity to store vitamin A, it is not indicated by alimentary vitaminuria.

Without exception a marked fecal loss of vitamin A occurred. That this loss was not entirely dependent on the intake is shown by the fact that two of the infants fed a diet very low in vitamin A excreted a larger amount than they ingested. The loss with different children on different diets varied from two to twelve per cent of the intake, the largest loss occurring with the largest intake. The greatest retention also occurred on the greatest intake. Since the fecal fat excretion of infants is normally within this range, it seems evident that the loss of vitamin runs parallel with the loss of fat.

The vitamin A of egg yolk and cod liver oil appear to be equally well utilized, but it was not conclusively shown whether the vitamin A of carrots was completely utilized, as the small amount the infants could take was too limited to make the results definitive.

The writer wishes to express her thanks to Dr. H. A. Mattill of the Department of Biochemistry for his constant interest and advice throughout this study and to the staff of the Department of Pediatrics for their suggestions and helpfulness and for their courtesy and kindness in furnishing materials and laboratory facilities.

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# THE DETERMINATION OF THE SURFACE AREA OF THE WHITE RAT

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Received for Publication—March 15, 1930

REGARDLESS of the theoretical basis of referring to metabolic rate measurements as functions of the surface area, experience has shown that the latter forms the most satisfactory basis for the expression of metabolic rates in various animals. Various ingenious methods have been used to determine formulae whereby the surface area may be predicted. Some of these methods have proven their value in certain types of animals, and for many species are well established. For the commonly employed rat, however, there is as yet no unequivocal method for predicting the surface area.

Various reports have been published setting forth formulae which are designed to give this result. They are all based on the original empirical formula postulated on a mathematical basis by Meeh in 1879. This formula $S = KW^{2/3}$ theoretically should be applicable to various objects when certain definite values of $K$ were known. The constant $K$, however, should theoretically vary for animals of the same type but of slightly different shape. To eliminate the factor of varying shape, several attempts have been made to introduce one or more linear measurements into the formula, but as yet this has not met with any success. The Meeh formula is for that reason still used in its original form.

Various values of $K$ for the rat have been reported by several workers. The earliest of these was Rubner who gave the value as 9.13 when the weight was in grams and the area in square centimeters. Pfaundler in 1916 said that the value was 9.74. No data on the number of animals nor the methods used in either case are available. Hill and Hill (1), on a series of five rats ranging in weight from 50 to 129 gms. reported the constant of 9.93. Their method is not described. Carmen and Mitchell (2) in 1926 published their results on a series of 62 rats and gave their constant as 11.36. This value, however, is open to criticism based on their method of procedure which consisted in measuring the rats, killing and skinning them. The skins were pinned out upon a board and the areas measured with a planimeter. Since the skin is an elastic organ which will vary widely in size according to the tension which is placed upon it, it follows that their

value is undoubtedly too large. Lee (3) has pointed out that there are strong reasons for believing that the skin was stretched by Carmen and Mitchell, since the heat production determined by indirect calorimetry when expressed as calories per square meter per hour of this skin was 24. This is at least 33 per cent less than the well established value for man and accordingly does not comply with the law of surface areas. Lee, in this same paper, gives a value of 9.00 for $K$, basing his result on the measurements of the varnished skins of 72 rats, males and females, whose weights varied from 19 to 418 grams. This value of $K$ makes the caloric output of the rat about 31 calories per square meter per hour, which is still some 16 per cent below that for man and other animals. Such a discrepancy suggests that his value of $K$ is also too high.

All the reports so far have been on so-called normal rats. Since in the rat the pattern of the various individuals is very nearly the same when they are kept under the same conditions, it does not seem as though an additional linear factor would be of any practical value in enhancing the accuracy of prediction. However, many animals used in the laboratory are of necessity going to live under varying conditions and it is conceivable that their body patterns are going to be altered. This holds true especially for emaciation and obesity.

These studies are the results obtained on two series of rats. The first series was composed of presumably normal animals, and the second was composed of emaciated animals.

In the choice of a method with which to gain necessary data we were confronted with the problem of finding a medium which would cover evenly and minutely all the irregularities of the body surface and which would peel off with ease. After many trials the following mixture was chosen because it gave a maximum of strength with a minimum shrinkage:

| | |
|---|---|
| Guncotton (dried) | 4 gms |
| Castor oil | 1.5 cc. |
| Turpentine | 2.0 cc. |
| Alcohol (95%) | 20.0 cc. |
| Ether | 80.0 cc. |

It was impossible to procure a mixture which showed no shrinkage whatever but the above is believed to be the most satisfactory. Repeated tests were made on various batches of this collodion to determine the amount of shrinkage. This was done by obtaining the measurements of hard rubber balls and then measuring the collodion film with which they

had been surrounded, using the same technique as was employed for the rats. The shrinkage values ran practically constant at 3.7 per cent, not varying more than one per cent from this. Similar results were obtained by covering metal objects.

The method consisted of weighting the rats, and then taking the following linear measurements when the animals were tied to a board: nose to anus; the girth in three places, namely, just behind the forelegs, mid abdomen, and just in front of the hind legs; the ear diameters, and the length and diameter of the base of the tail. The animals were then chloroformed and placed in a small amount of a mixture of powdered barium sulfide and weak sodium hydroxide. This was sufficient to remove the hair in from five to fifteen minutes. They were then washed clean, dried, and covered with a very thin coat of vaseline. They were dipped in the collodion mixture, allowed to drain, and were dried in a current of air until the film could be touched without sticking. The process was repeated and the double-layered film was allowed to dry not over twenty minutes.

The dried film was peeled off by making dorsal and ventral longitudinal incisions and one single cut along each leg. The pieces so procured were cut up into a number of smaller ones (from 10 to 20), so that they would lie flat. They were then pressed under a warm electric iron and measured with a planimeter. Each set of pieces was checked twice with this instrument with a maximum allowable error of 0.3 per cent on each series of pieces measured.

The films so obtained were found to conform to body irregularities with remarkable accuracy and, by cutting the pieces smaller, there could be obtained on a flat surface the exact area of the body. In order that the process of peeling go smoothly the hair must be completely removed and the vaseline coating be extremely thin.

The animals used in the experiments were selected from the laboratory stock in such a manner as to get the widest possible variety of types. Those which were used for the starvation experiments were placed in cages and food was withheld. They were allowed an unlimited quantity of water, and the starvation was continued as long as was feasible (usually from 3 to 5 days). Care was taken to use no animals which had died and showed any signs of post mortem changes such as rigor or bloating of the abdomen.

In calculating the results two assumptions were made, the validity of which was proved by checking experiments. The ears were measured in two diameters and were calculated as perfect circles since these two values

TABLE I

THE VALUES OF $K$ FOR THE RAT IN VARIOUS FORMULAE FOR THE SURFACE AREA

| Body wt. $W$. gm. | Body length $L$. cm. | Girth $G$. cm. | Actual surface area $S$. sq. cm. | $K$ $S=KW^{\frac{2}{3}}$ | $K$ $S=K\sqrt{W^{\frac{2}{3}}LG}$ | $K$ $S=KLG$ |
|---|---|---|---|---|---|---|
| | | | Untreated Stock Animals | | | |
| 24.5 | 10.0 | 5.8 | 62.5 | 7.44 | 2.874 | 1.078 |
| 25.5 | 10.5 | 6.4 | 65.8 | 7.63 | 2.785 | 0.980 |
| 38.5 | 11.7 | 7.1 | 81.2 | 7.12 | 2.695 | 0.979 |
| 41.0 | 12.0 | 7.2 | 83.0 | 6.98 | 2.650 | 0.961 |
| 42.0 | 12.3 | 7.2 | 87.8 | 7.30 | 2.742 | 0.991 |
| 51.5 | 13.0 | 8.5 | 97.6 | 7.07 | 2.545 | 0.885 |
| 53.0 | 12.7 | 7.9 | 109.5 | 7.75 | 2.940 | 1.092 |
| 60.0 | 13.7 | 8.5 | 110.7 | 7.25 | 2.680 | 0.928 |
| 64.0 | 13.6 | 7.4 | 106.2 | 6.71 | 2.706 | 1.058 |
| 64.0 | 14.3 | 8.2 | 119.1 | 7.52 | 2.805 | 1.019 |
| 66.0 | 14.3 | 8.3 | 122.7 | 7.52 | 2.840 | 1.032 |
| 68.5 | 14.5 | 8.6 | 118.9 | 7.16 | 2.660 | 0.953 |
| 69.0 | 15.2 | 8.3 | 124.5 | 7.41 | 2.758 | 0.999 |
| 69.0 | 14.5 | 8.2 | 128.4 | 7.59 | 2.898 | 1.082 |
| 70.0 | 14.8 | 8.5 | 123.2 | 7.26 | 2.720 | 0.980 |
| 71.0 | 14.5 | 9.1 | 111.4 | 6.51 | 2.365 | 0.843 |
| 71.5 | 14.6 | 8.8 | 115.0 | 6.72 | 2.480 | 0.896 |
| 73.0 | 14.7 | 9.4 | 124.1 | 7.13 | 2.633 | 0.897 |
| 79.5 | 14.2 | 9.4 | 138.1 | 7.29 | 2.832 | 1.037 |
| 81.5 | 15.4 | 9.0 | 134.8 | 7.15 | 2.700 | 0.967 |
| 88.7 | 16.0 | 9.5 | 135.2 | 6.84 | 2.500 | 0.890 |
| 92.0 | 15.0 | 9.1 | 130.4 | 7.54 | 2.741 | 0.956 |
| 95.5 | 16.0 | 9.4 | 162.0 | 7.73 | 2.925 | 1.078 |
| 100.0 | 18.2 | 9.8 | 172.4 | 8.05 | 2.833 | 0.966 |
| 101.5 | 17.0 | 9.8 | 158.3 | 7.29 | 2.690 | 0.950 |
| 103.0 | 16.5 | 10.2 | 147.8 | 6.76 | 2.470 | 0.879 |
| 110.0 | 16.6 | 9.8 | 156.6 | 6.83 | 2.620 | 0.961 |
| 114.0 | 17.6 | 10.6 | 178.0 | 7.61 | 2.745 | 0.955 |
| 114.5 | 17.0 | 10.3 | 167.7 | 7.16 | 2.670 | 0.958 |
| 122.0 | 17.3 | 10.3 | 191.5 | 7.82 | 2.928 | 1.072 |
| 130.5 | 17.3 | 10.6 | 186.5 | 7.29 | 2.770 | 1.017 |
| 134.5 | 18.0 | 11.5 | 185.4 | 7.06 | 2.565 | 0.896 |
| 137.5 | 18.2 | 11.4 | 182.3 | 6.87 | 2.490 | 0.880 |
| 142.0 | 18.5 | 11.0 | 197.7 | 7.34 | 2.715 | 0.970 |
| 144.0 | 18.0 | 10.9 | 197.2 | 7.19 | 2.745 | 1.005 |
| 144.5 | 18.5 | 10.9 | 193.8 | 7.03 | 2.660 | 0.961 |
| 146.0 | 17.0 | 11.4 | 204.8 | 7.47 | 2.840 | 1.058 |
| 151.5 | 19.0 | 11.2 | 217.4 | 7.65 | 2.845 | 1.020 |
| 152.0 | 18.5 | 11.8 | 208.4 | 7.35 | 2.700 | 0.957 |

TABLE I. (*continued*)

| Body wt. *W.* gm. | Body length *L.* cm. | Girth *G.* cm. | Actual surface area *S.* sq. cm. | $K$ $S=KW^{\frac{2}{3}}$ | $K$ $S=K\sqrt{W^{\frac{2}{3}}LG}$ | $K$ $S=KLG$ |
|---|---|---|---|---|---|---|
| 154.5 | 19.5 | 11.8 | 210.7 | 7.31 | 2.650 | 0.916 |
| 163.5 | 18.0 | 11.9 | 199.8 | 6.72 | 2.545 | 0.934 |
| 167.0 | 18.5 | 11.8 | 222.0 | 7.35 | 2.784 | 1.017 |
| 167.5 | 19.0 | 11.6 | 227.6 | 7.23 | 2.831 | 1.032 |
| 168.0 | 19.6 | 12.1 | 208.8 | 6.89 | 2.500 | 0.881 |
| 172.5 | 19.5 | 11.6 | 231.1 | 7.48 | 2.788 | 1.021 |
| 177.0 | 20.5 | 11.7 | 240.2 | 7.66 | 2.812 | 1.002 |
| 193.0 | 19.0 | 12.2 | 232.0 | 6.98 | 2.698 | 1.000 |
| 195.0 | 21.0 | 12.5 | 244.8 | 7.32 | 2.670 | 0.935 |
| 196.0 | 21.0 | 12.4 | 241.6 | 7.19 | 2.632 | 0.926 |
| 197.0 | 20.5 | 11.9 | 253.8 | 7.51 | 2.842 | 1.038 |
| 210.0 | 20.5 | 13.2 | 262.6 | 7.38 | 2.745 | 0.972 |
| 218.5 | 20.0 | 13.4 | 231.9 | 6.39 | 2.375 | 0.865 |
| 228.0 | 21.5 | 13.4 | 270.5 | 7.29 | 2.669 | 0.934 |
| 237.0 | 20.5 | 13.5 | 258.8 | 6.99 | 2.565 | 0.935 |
| 261.0 | 22.0 | 13.5 | 287.9 | 7.10 | 2.675 | 0.970 |
| 366.5 | 25.0 | 15.5 | 352.7 | 6.93 | 2.555 | 0.912 |
| Mean: | | | | 7.20 | 2.71 | 0.914 |
| Standard deviation of the percentage errors: | | | | 4.84% | 5.18% | 6.34% |
| Starved Animals | | | | | | |
| 22.0 | 10.5 | 6.1 | 58.3 | 7.41 | 2.600 | 0.911 |
| 44.0 | 13.6 | 8.5 | 92.4 | 7.38 | 2.425 | 0.800 |
| 59.0 | 14.5 | 9.5 | 105.5 | 6.99 | 2.280 | 0.767 |
| 77.0 | 16.6 | 10.2 | 140.8 | 7.72 | 2.542 | 0.831 |
| 87.5 | 18.1 | 9.1 | 165.7 | 8.45 | 2.870 | 1.109 |
| 91.0 | 17.0 | 9.1 | 149.8 | 7.44 | 2.672 | 0.968 |
| 96.6 | 17.0 | 10.2 | 153.4 | 7.33 | 2.540 | 0.885 |
| 99.0 | 17.7 | 9.9 | 156.5 | 7.35 | 2.558 | 0.894 |
| 101.0 | 17.9 | 9.6 | 162.1 | 7.55 | 2.652 | 0.944 |
| 102.0 | 18.1 | 11.2 | 154.3 | 7.06 | 2.295 | 0.763 |
| 114.0 | 18.9 | 10.2 | 178.6 | 7.60 | 2.650 | 0.926 |
| 127.5 | 18.5 | 10.7 | 181.9 | 7.19 | 2.570 | 0.919 |
| 150.0 | 20.0 | 11.9 | 198.1 | 7.07 | 2.408 | 0.839 |
| 164.0 | 19.5 | 12.2 | 208.4 | 6.96 | 2.465 | 0.879 |
| Mean: | | | | 7.37 | 2.542 | 0.877 |
| Standard deviation of the percentage errors: | | | | 4.74% | 5.83% | 8.29% |

were practically the same. Both sides of each ear were taken into account. The tail, which formed a convenient handle with which to manipulate and dry the animals, was calculated as being a perfect right cone, the

length and diameter of the tail being the necessary factors in the calculation. This latter assumption proved to be allowable by covering the organ with the collodion mixture and measuring the actual area. The ear and tail areas were added to the film area.

TABLE II

THE VALUE OF $K$ WHEN VARIOUS EXPONENTS OF $W$ ARE USED

| Exponents | $K$ | Standard deviation of percentage errors | Within 5 Per cent |
|---|---|---|---|
| $x$ | $\frac{S}{W^x}$ | Per cent | Per cent |
| | Untreated | | |
| 0.58 | 10.80 | 6.49 | 46.4 |
| 0.60 | 9.84 | 5.74 | 58.9 |
| 0.62 | 8.96 | 5.18 | 62.5 |
| 0.64 | 8.16 | 4.85 | 71.5 |
| 0.66 | 7.43 | 4.79 | 69.6 |
| 0.667 | 7.20 | 4.84 | 71.5 |
| 0.68 | 6.77 | 5.03 | 66.1 |
| | Starved Rats | | |
| 0.58 | 10.84 | 5.82 | 71.5 |
| 0.60 | 9.92 | 5.23 | 78.5 |
| 0.62 | 9.07 | 4.86 | 78.5 |
| 0.64 | 8.30 | 4.63 | 85.7 |
| 0.66 | 7.59 | 4.68 | 71.5 |
| 0.667 | 7.37 | 4.74 | 78.5 |
| 0.68 | 6.94 | 4.92 | 64.3 |

With this information at hand, the value of $K$ was obtained in different formulae. Table I shows the value of $K$ using the Meeh formula, that is, when the surface area equals the two-thirds power of the weight multiplied by a constant. It will be seen that the mean value for $K$ in untreated animals is 7.20 and for the starved animals is 7.37. The constant in 72 per cent of the cases for the untreated animals falls within $\pm 5$ per cent of the mean, while for starved animals 79 per cent fall within $\pm 5$ per cent of the mean.

Table I also shows the value of $K$ in the formula $S = K\sqrt{W^{2/3}LG}$.

The girth was taken as an average of the three measurements, namely: just behind the fore legs, mid abdomen, and just in front of hind legs. It will be seen that this is a less satisfactory way of obtaining $K$ since in only 66 per cent of the untreated animals and 64 per cent of the starved ones is the value within $\pm 5$ per cent.

Table I shows likewise the value of $K$ in the formula $S = KLG$. This is clearly a poor method for obtaining $K$ since distinctly less than 50 per cent of the values for it are within $\pm 5$ per cent.

TABLE III

THE HEAT PRODUCTION OF VARIOUS ANIMALS REFERRED TO THEIR SURFACE AREAS

| Animal | Heat production. Calories per sq. meter per hour | Remarks |
|---|---|---|
| Horse | 39.5 | |
| Pig | 45.2 | |
| Man | 35—40 | |
| Dog | 43.2 | |
| Rabbit | 32.4 | Including ears. |
| Rabbit | 38.2 | Without ears. |
| Goose | 40.4 | |
| Fowl | 39.3 | |
| Mouse | 49.5 | |
| Rat | 24.0 | Carman & Mitchell's constant of 11.36 |
| Rat | 30.2 | Lee's constant of 9.00 |
| Rat | 36.5 | "Constant arrived at in this paper: 7.47" |

It remains to be seen whether the first formula $S = KW^{2/3}$ might be improved if some other exponent of $W$ were used. Table II shows the results for different exponents. It will be seen that the exponent 0.667 gives as reliable results as any other exponent. Accordingly no improvement of the fomula $S = KW^{2/3}$ can be made by changing the exponent.

The Meeh constants for the untreated and starved animals differed by only 2.3 per cent. It will be recalled that the collodion films showed a shrinkage of 3.7 per cent. Hence, the determined value of $K$ should be increased by 3.7 per cent. For the untreated group this value of $K$ becomes 7.47 and for the starved animals 7.64. The difference in these two constants is extremely small and may be explained by the fact that a smaller series of starved animals was used. Since this difference is negligible it is clear that the constant 7.47 may apply equally well to both normal and emaciated animals.

This value is approximately 17 per cent lower than that given by Lee. That it probably approaches the true value more nearly than does 9.00 may be demonstrated by the fact that when the value of 7.47 is used as the constant in the Meeh formula, the heat production of the rat becomes 36.5 calories per square meter per hour. This value was obtained by correcting Lee's figures as follows: For any given rat the surface area would be decreased by 17 per cent if this constant were substituted for that of Lee. Consequently the heat production per square meter for such a rat would be increased by 17 per cent. This is far more comparable with the values for other animals than the value of 30.2 calories calculated from Lee's formula.

Though the heat production of various animals has not been shown to be identical, they all have been shown to have higher values than that calculated by Lee for the rat. The figures as calculated from Meeh's formula and as given by Lusk (4) are found in Table III. From this table it can be seen that the heat produced by the rat is quite comparable with that of other species when based on an area computed by using the formula $S = 7.47W^{2/3}$.

## Summary

A series of 56 normal rats and a series of 14 starved rats were weighed, measured, and their surface areas determined by measuring a collodion film which had been applied to the depilated skin. The data obtained were used to construct a formula for the prediction of the surface area of the rat. The best results were obtained by using the formula $S = 7.47W^{2/3}$ for the normal animals and $S = 7.64W^{2/3}$ for the starved ones. The standard deviations of the percentage errors of the above are 4.84 per cent and 4.74 per cent respectively.

By using the constants given above the heat production of the rat referred to the surface area is more comparable to that of other animals than when older constants were used in the area prediction.

I wish to express my appreciation to Dr. L. H. Newburgh for his suggestions and help in going over the data. I also wish to thank Mr. H. C. Carver and his staff for their assistance in the calculations.

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# GLYCOGEN FORMATION IN RATS

## II. DIETS CONTAINING APPROXIMATELY EIGHTY-SEVEN PER CENT OF THE TOTAL CALORIC VALUE IN THE FORM OF SUCROSE, LARD, AND CASEIN, RESPECTIVELY

*By*

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Received for Publication—March 17, 1930

IN THE first paper (1) of this series, we presented results found by feeding young rats diets containing approximately 60 per cent of the total caloric value in the form of starch, sucrose, lard and casein.

In the present study we have used more extreme diets and have found more striking differences. The control diet was McCollum's Stock Diet I (whole ground wheat 67.5 per cent, casein 15, whole milk powder 10, butter fat 5.2, calcium carbonate 1.5 and sodium chloride 0.8). This diet has a value of about 3.88 Calories per gram (not considering digestibility), of which 25.4 per cent is derived from protein, 20.3 per cent from fat, and 54.3 per cent from carbohydrate.

The composition of the test diets is shown in tabular form, the figure in parenthesis representing the per cent of the total caloric value derived from each constituent (not considering digestibility).

| Diet | Casein | Sucrose | Lard | Salt (185) | Cal. per gm. |
|---|---|---|---|---|---|
| A | 14.5 (13.1) | 81.6 (86.9) | 0 | 3.9 | 3.77* |
| B | 30.0 (12.4) | 0 | 80 (87.6) | 8.2 | 6.95 |
| C | 95.0 (87.8) | 0 | 5 (12.2) | 4.2 | 3.53 |

We allowed 1 gm. of McCollum's "salt mixture 185" per 100 Calories, as suggested by Dr. Burr (2). Each test diet was supplemented by vitamin feeding, as in the preceding study.

Adult rats were used. The breeding stock which had served during the year, and all of the healthy discards we could secure from Dr. C. M. Jackson, were employed. The rats were kept on the stock diet for observation during two to three weeks, and then placed on the test diets for a

* Dr. G. O. Burr found that our crude casein contains about 85 per cent casein, so this figure has been used in calculating the caloric value.

period of about three weeks (18–23 days). The food consumption and the water intake were measured during both the preliminary and the test periods. At the end of the test period, the rats were killed (without any starvation period), and the glycogen determinations carried out as described in the previous paper.

## Results

The combined results for males and females are shown in Table I. The rats on each diet showed a fair gain in weight during the test period.

Table I

Average Weights, Food Consumption and Water Intake

| Diet | Number Sex | Weight | | | Daily Food Intake | | | Daily Water Intake | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Begin. test period | Final | Gain test period | Stock diet | Test diet | Diff. in calorie intake | During stock diet | During test diet | Diff. |
| | | gms. | gms. | gms. | gms. | gms. | | cc. | cc. | cc. |
| Stock (Control) | 10 {6F 4M | 250.6 | 262.6 | 12.0 | 12.5 | 12.8 | +1.16 | 19.3 | 20.0 | +0.7 |
| Sucrose A | 13 {9F 4M | 258.8 | 277.7 | 18.9 | 11.3 | 12.7 | +4.04 | 23.5 | 15.4 | −8.2 |
| Lard B | 13 {9F 4M | 222.6 | 242.7 | 20.1 | 11.6 | 7.0 | +3.64 | 18.9 | 12.7 | −6.2 |
| Casein C | 12 {9F 3M | 206.0 | 220.3 | 14.3 | 12.4 | 12.5 | −3.99 | 23.2 | 29.8 | +6.6 |

If the gain be expressed in terms of per cent of the initial body weight, the control animals gained 4.8 per cent, those on sucrose 7.3, on lard 9.0, and on casein 6.9 per cent. These figures indicate that adjustment had taken place during the test period.

Since we know neither the digestibility of the various diets, nor the individual absorptive powers of the rats, it is difficult to determine the significance of the slight increase in caloric intake on the sucrose and lard diets, and the comparable decrease on the casein diet.

The differences in water intake were interesting. The rats on the sucrose diet showed an average decrease of 34.9 per cent in the daily water intake, which surprised us greatly. The 32.8 per cent decrease on the lard diet was expected. Osborne (3) called attention to the increase in water

intake on a high protein diet, but no figures were given. Our increase on the casein diet amounted to 28.4 per cent.

The liver weights and glycogen contents are shown in Table II. In order to make the desired comparisons between the control and the test diets, we have used the control value for the subtrahend in every case. The

TABLE II

LIVER WEIGHTS AND GLYCOGEN CONTENTS

| Diet | Av. liver wt. | Liver wt. as per cent of body wt. | Per cent of liver glycogen | Liver glycogen per gm. body wt. |
|---|---|---|---|---|
| | gms. | | | mgms. |
| Stock (Control) | 9.12±0.72 | 3.487±0.096 | 4.49±0.19 | 1.578±0.083 |
| Sucrose A | 9.69±0.35 | 3.572±0.063 | 6.03±0.18 | 2.182±0.065 |
| Lard B | 7.43±0.38 | 3.078±0.057 | 1.79±0.12 | 0.543±0.034 |
| Casein C | 8.53±0.35 | 3.863±0.058 | 2.02±0.15 | 0.796±0.069 |

differences are shown in Table III. There was no significant difference in absolute liver weight between the stock diet and any one of the test diets. However, if liver weight be expressed as per cent of total body weight, we find that the high lard diet gave a significantly lighter liver, while the high casein diet gave a significantly heavier one than the stock

TABLE III

DIFFERENCES BETWEEN TEST AND CONTROL ANIMALS

| Diet | Differences in liver weights | | | | Differences in glycogen contents | | | |
|---|---|---|---|---|---|---|---|---|
| | gms. | Diff. P.E. diff. | Per cent of wt. | Diff. P.E. diff. | Per cent | Diff. P.E. diff. | Mgms. per gm. body wt. | Diff. P.E. diff. |
| Sucrose | +0.57±0.80 | 0.7 | +0.085±0.11 | 0.8 | +1.54±0.26 | 5.9 | +0.604±0.10 | 6.0 |
| Lard | −1.69±0.81 | 2.1 | −0.409±0.11 | 3.7 | −2.70±0.22 | 12.3 | −1.035±0.090 | 11.5 |
| Casein | −0.59±0.80 | 0.7 | +0.376±0.11 | 3.4 | −2.47±0.24 | 10.3 | −0.782±0.11 | 7.1 |

diet. Of course the wet weight of the liver is given and a variation in water content may be responsible for a part of these differences.

As might be expected, the high sucrose diet gave a significantly greater glycogen content than the control diet. The high lard and high casein diets produced a significantly lower glycogen content. This relationship holds whether the glycogen is expressed as per cent, or as milligrams per

gram of body weight. These differences are much more striking than those found on the 60 per cent diets reported previously.

During the last 24 hours the food intake was measured in two twelve-hour periods. One extended from 8:30 A.M. to 8:30 P.M., and the other from 8:30 P.M. to 8:30 A.M., when the rats were killed, without any starvation period. As expected, all the rats ate more during the night period. Of the total final 24 hour intake, the control rats ate 67.1 per cent during this time (8:30 P.M. to 8:30 A.M.). The figures for the other diets were: sucrose 60.3, lard 63.6 and casein 67.6 per cent.

We wished to determine whether the per cent of glycogen was dependent upon the average daily intake of food, upon the final 24 hour intake, or upon the final 12 hour intake. The usual statistical methods were employed in deriving the information. This relationship between food intake in grams and glycogen percentage on the stock diet may be expressed by the following correlation coefficients; for the average daily intake $+0.68 \pm 0.16$, for the final 24 hour intake $+0.82 \pm 0.10$, and for the final 12 hour intake $+0.93 \pm 0.04$. The significance of this relationship will be evident by dividing the correlation coefficient by its probable error. A quotient of 2.5 represents the lower limit of significance, and the higher the quotient, the more significant the relationship becomes. The quotients in the three cases above are 4.2, 8.2, and 23.2. We may conclude that the glycogen content in rats on the stock diet is determined largely by the amount of food eaten during the last twelve hours.

The same means of calculation applied to the sucrose results showed an absence of correlation between glycogen content and food intake. We believe that this absence of correlation indicates that rats on a high sucrose diet have livers which are saturated with glycogen. If the livers are practically saturated all the while, obviously the amount of food eaten during the last twelve hours cannot directly determine the glycogen percentage.

The high lard diet gave results which indicated a lack of correlation between food intake and glycogen content. On such a diet, glycogen is formed in an irregular manner, consequently a direct relationship is not to be expected.

That a slight relationship exists between the final 12 hour food intake (in grams) and glycogen content on a high casein diet, is shown by a correlation coefficient of $+0.53 \pm 0.17$. We may conclude either that it is easier to form glycogen from casein than from lard, or that the small amount of lactose present as an impurity in the crude casein is utilized for the purpose.

In order to gain further information from our results, we have determined the caloric intake during the last twelve hours. In order to rule out the variations in body weight, we expressed the intake as Calories per gram of body weight. The glycogen was expressed as milligrams per gram of liver. Again using the ordinary statistical formulae, we derived the following correlation coefficients; stock diet $+0.55 \pm 0.21$, sucrose diet $-0.30 \pm 0.23$, lard diet $-0.44 \pm 0.19$, and casein diet $+0.50 \pm 0.18$. In other words, the rats on the stock and casein diets showed a significant correlation between caloric intake during the last twelve hours and glycogen content. The results for the sucrose and lard diets were not significant. It will be noted that the relationship between caloric intake and glycogen is less marked on the stock diet than was that between the intake in grams and the glycogen content. Perhaps the digestibility of the stock diet could clear up this question.

We found the interesting fact that an average intake of 0.1511 Cal. per gram of body weight corresponded to an average of 46.54 mgms. of glycogen per gram of liver on the stock diet, on the sucrose diet 0.1148 Cal. = 65.29 mgms. of glycogen, on the lard diet 0.1409 Cal. = 19.23 mgms. of glycogen, and on the casein diet 0.1127 Cal. = 21.19 mgms. of glycogen. This led us to work out an "efficiency" table, with respect to glycogen formation on the different diets. If we represent the efficiency of glycogen formation (in relation to caloric intake) of the stock diet by 1, then the relative efficiency of the sucrose diet is 1.85, of the lard diet 0.44 and of the casein diet 0.61.

The rats on the test diets were receiving vitamins daily, as before mentioned. The vitamin mixture was given at 4 P.M., including the day before killing. Just what effect this feeding may have had on the results has not been determined. We hope to continue the investigations on glycogen formation with metabolic studies in addition.

## Summary and Conclusions

1. After a preliminary observation period on McCollum's stock diet I, adult rats were fed test diets containing about 87 per cent of the total caloric value in the form of sucrose, lard or casein, for about three weeks. The food and water intakes were determined during both the preliminary and the test period.

2. The gain in body weight was evident on each diet suggesting adaptation.

3. Without knowing the digestibility of each diet, and the absorptive

power of each rat, it is difficult to interpret the changes in caloric intake during the test period.

4. The water intake increased on the high casein diet, and decreased on the lard and sucrose diets. This is probably determined by the amount of nitrogenous waste products to be eliminated.

5. The absolute liver weights were in close agreement on the various diets. Considered as per cent of body weight, the liver was lighter on the high lard diet and heavier on the high casein diet than on the control diet. Dry weights could not be determined.

6. Liver glycogen was significantly higher on the high sucrose and significantly lower on the lard and the casein, than on the control diet.

7. An exceedingly close relationship was found between the last twelve-hour food intake (grams) (8:30 P.M. to 8:30 A.M.) and the liver glycogen content, on the stock diet.

8. The absence of such close agreement between late food intake and glycogen content on the sucrose diet suggested that in a liver already saturated with glycogen, more cannot be stored.

9. Glycogen is probably not readily formed on a high lard diet, consequently it did not vary in accordance with the late food intake.

10. There was some relation between the late food intake and glycogen content on the high casein diet. Either it is easier to form glycogen from casein, or the small amount of lactose present as an impurity in the crude casein is utilized.

11. From the late intake, expressed as Calories per gram of body weight, and the glycogen content expressed as milligrams per gram of liver weight, we have worked out a glycogen formation efficiency scale. If our stock diet possesses an efficiency of unity, the values are 1.85 for sucrose 0.44 for fat and 0.61 for casein.

We desire to express our indebtedness to Dr. C. M. Jackson, Dr. G. O. Burr and Dr. F. H. Scott for their kindly interest and criticism.

A grant from the Medical Research Fund helped to defray a part of the cost of this study.

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# MEAT IN NUTRITION

## I. PRELIMINARY REPORT ON BEEF MUSCLE*

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Received for Publication—March 28, 1930

### INTRODUCTION

MEAT, like milk, has been subjected to many and varied tests in order to determine its rôle in nutrition. The earlier experiments, such as those of Watson (1), conducted before the formulation of the vitamin hypothesis are not comparable with experiments of to-day because the diets used were obviously inadequate or one-sided in the light of our present conception of an animal's nutritive needs. More recent researches have taken the rôle of the vitamins into consideration and the rations have been planned to provide the accessory factors in so far as they are known.

However, much of the recent work may be criticized because only portions of the life cycles of the experimental animals were studied. It was McCollum and Davis (2) who pointed out that "even normal growth to the normal adult size and continued maintenance does not necessarily indicate perfect nutrition. Only when animals reproduce and nourish their young normally and repeat this at normal intervals, can it be said that the ration is physiologically sufficient." Simmonds (3), also, has observed that "any fault in the diet, however slight, which causes it to deviate from the optimum, will, if it exerts its influence over a sufficient period of time, surely be reflected somewhere in the life history of the individual or its descendants."

Palmer and Kennedy (4) emphasize the importance of procuring with regularity the growth, reproduction, lactation, and longevity responses of animals on various diets before we can hope to have adequate knowledge of the fundamental needs for each important biological period.

Hoagland and Snider (5), however, feel that the method of determining the relative values of different proteins for all body functions by a series of feeding experiments extending through several generations is open to criticism. A single protein may not be equally efficient for maintenance,

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or repair of body tissue and as a constituent of internal secretions; for growth; for reproduction, and for lactation. Their growth experiments with rats, therefore, deal only with 30-day and 60-day periods.

This paper describes the growth, reproduction and lactation performances of three generations of albino rats living on a diet in which the main protein constituent was lean muscle beef. One object of the project was to ascertain what level of muscle beef, when adequately supplemented with the known dietary factors, would provide the minimum level of beef protein necessary for normal growth, reproduction, and lactation. After the lowest level of muscle beef protein had been ascertained, it was planned to feed an intermediary level of protein, then an excessively high level and to observe the effects of such intake on the animals through not less than five generations. The original plan of work has had to be modified, however, because the results were not such as to warrant continuing the experiment as outlined. A diet adequate for the rat during the period of growth and reproduction may not necessarily be adequate during the critical period of lactation. If a diet fails to support lactation it must be so modified as to remedy the deficiency or otherwise it cannot be termed properly adequate.

## Historical

The literature reviewed is pertinent because muscle beef was used to form at least a portion of the protein of the diets reported therein, but is not comparable in every instance because only portions instead of the entire life cycles of the rats were studied. In a study of the rate of growth and voluntary activity of rats, Hitchcock (6) added daily 8 grams of fresh lean meat cooked rare to a basal diet affording approximately 27 per cent of protein, quite largely milk proteins, and followed the growth and activity of the rats from 30 to 390 days. He reports that the rats receiving the meat grew more rapidly and weighed more at maturity than the controls without meat, but that the differences in voluntary activity were not pronounced and hence were inconclusive. The meat protein very evidently supplemented the milk proteins to a measurable extent.

Desiring to ascertain at what age an increased growth resulting from meat feeding could be produced, Hitchcock (7) fed mother rats meat, 8–10 grams daily, beginning the day the young were born. He increased the daily feedings of meat as the young became older until each baby rat received a gram or two of meat daily. The young were continued on the meat diet until maturity, then bred, and their young became the second generation. The experiment was carried through three generations. There were in all, eleven control and thirteen meat-fed litters. The results favor the meat-fed mothers in contrast to the controls without meat. The basal ration was the same as that used in the previous study, wherein milk proteins and whole wheat flour proteins provided 27 per cent of the total protein. Here again, the meat protein proved a desirable supplement to the milk proteins in an otherwise adequate diet. But are the results of such experiments wherein the meat used forms only a part of the protein of the ration, a true picture of the rôle of meat in nutrition?

MacLeod (8) fed fresh, raw, lean beef, from 10 to 40 grams per week to one group of rats and dried beef from 3 to 12 per cent of the dry food mixture to a second group. As in Hitchcock's experiments, the meat was fed in addition to the basal diet which contained 12 per cent of whole milk powder and 30 per cent of patent flour and which was fortified with vitamin-containing foods.

The addition of the raw beef raised the proportion of protein from 9.9 to about 17.5 per cent. The addition of the dried meat changed the protein content to 14.9 per cent. The growth of the animals on both the liberal and low meat diets was normal. Reproduction and lactation were improved on the diet having the 17.5 per cent of protein. When the dried meat was made a part of the diet instead of the fresh meat, reproduction was not so good, yet reproduction and lactation were somewhat benefited by the increased protein content of the dried meat diet. Reproduction and lactation were best on the 14.9 per cent level of protein. When the basal diet was improved by increasing the percentage of milk from 12 per cent to 16 per cent there was successful reproduction on both 3 per cent and 12 per cent of dried meat. A greater number of young of higher weight were reared on the diet containing 12 per cent of meat.

Simmonds (9) reports reproduction of rats on synthetic diets in which cooked round steak as 94.5, 80.5 and 50 per cent of the diet supplying 67, 57, and 35 per cent of protein respectively, was used. Lactose or dextrin replaced a part of the meat in the diets when the protein supplied was less than 67 per cent. Salts and butterfat were included also, so the diets were seemingly adequate except for their high protein content. Observations of growth, fertility and infant mortality were made. The animals were not kept on these diets their entire life span, however, so the records must be viewed with this fact in mind. The mothers were fertile and on the whole had good success in rearing their young. The exceptions were when the mother destroyed the young very shortly after birth.

Muscle of ox was fed by McCollum, Simmonds and Parsons (10) to rats as the sole source of protein for growth and reproduction. The ration was made up to contain 9 per cent of protein (N×6.25) and adequate quantities of the vitamins A and B and mineral matter. Growth, reproduction and appearance of the rats were judged through second and third generations. The muscle of ox was shown to possess about the same biological value as the protein of the wheat kernel.

Hoagland and Snider (5) tested the nutritive value of the protein of voluntary muscle, and of heart and kidney from the ox, sheep and hog. The meat proteins were incorporated into the rations in such proportions that they contained 10, 12.5 and 15 per cent of protein (N×6.25). Round steak from fat steers was the ox muscle used. They concluded that as far as the protein from these three animals was concerned, it seemed likely that when the diet contained an adequate amount of protein these tissues would have approximately the same value for maintenance and growth.

Because of the variations in objectives and technic used in the experiments referred to, comparison of the results of one investigator with those of another is extremely difficult. In the experiments reported in this series of papers, the nutritive value of muscle beef was tested by making it the main protein constituent of a "synthetic" diet well fortified with vitamins and mineral matter. The animals were continued upon the diet until several generations had been observed.*

## Experimental Procedure

Albino rats were used as the experimental animals. The parent generation of two females and one male for each group was started on the meat and control rations when they were about 56 days of age. The young from

* Since the above was submitted for publication two articles by Clayton, M. M., (This Journal, 1930, II, 491, and III, 23), have appeared. A comparison of beef muscle with other animal proteins for growth, reproduction and lactation in albino rats was made. The basal rations were supplemented to provide the food constituents considered necessary for reproduction and lactation. Growth on the beef muscle ration was normal or better without exception,—results similar to ours. Both reproduction and lactation were unsuccessful, however. Lard was the fat used whereas we used butter fat throughout.

litters one and two were reared and became the first generation of rats on the test diets. The young were reared in large cages and the sexes not separated. As they neared maturity the females were weighed more often and when pregnancy was recognized by rapidly increasing weight, they were transferred to separate cages.

The rats were housed in all-metal, raised-bottom cages and handled according to the directions of Ferry (11). They were fed a basal ration consisting of pressure cooked lean beef* 50, cornstarch 30, dried brewery yeast 5, salt mixture (12) 5, butterfat 8, cod liver oil 2, and sodium chloride 1. The meat was ground in a meat chopper and all the ingredients well mixed to prevent selection by the rats. There was enough moisture in the meat to give the finished ration a plastic consistency. The ration contained a total crude protein content of 14.2 per cent (Nx6.25). The basal diet and water were fed *ad libitum*.

The acid and base potentiality of the basal diet was calculated and found to be 0.167 cc. N base per gram of the diet. Watson (1) attributed the results he obtained in his work, partly to the acid nature of the all meat ration used, and partly to the calcium deficiency.

The ratio of calcium to phosphorus in the basal ration was determined. It was found to be 0.5669 gram calcium to 0.4556 gram phosphorus per 100 gram of ration. Simmonds (9) states that the rat requires about 0.64 gram of calcium per 100 grams of food for optimal well being. In a summary reported by her of five diets made up of a mixture of grain proteins with 10 per cent of casein and having a total of 21 per cent of total protein, the rations varied in their ratio of calcium to phosphorus from 0.013 gram to 0.263 gram of calcium to 0.397 gram of phosphorus per 100 grams of ration. The phosphorus was kept constant and the calcium varied in quantity. There was 2 per cent of cod liver oil in the ration. The results obtained in growth, fertility and lactation, with the rats consuming these rations, were thought to be good.

According to Grieves (13) 0.41 gram phosphorus per 100 grams of ration is optimal for phosphorus. He found "good growth, well being, fertility and normal osseous tissues were induced in the rat when the relation of the total calcium was 0.64 and phosphorus 0.41 to 100 grams by weight, with butter fat at 2.5 per cent and cod liver oil at 1.5 per cent of the total diet representing the organic factors provided the other dietary elements were correct."

* The fresh round of beef, secured in quantity, was processed at 10 to 15 pounds pressure for 60 minutes, sealed in tin cans and used as needed.

The basal ration was supposedly amply reinforced with vitamins, the vitamins A and D being furnished by the butter fat 5, and cod liver oil 2, vitamin B in the yeast 5, and the vitamin E in traces in the meat. Vitamin C is thought to be synthesized by the rat and, hence, a source of it in the diet is supposedly unnecessary.

The control rats were fed a dog biscuit* and water *ad libitum*, with supplements of 30 cc. of whole milk daily and an occasional feeding of green food, usually lettuce. The dog biscuit was composed of water 8.2, animal protein 11.3, nitrogen free extract 57.55, calcium .51, ash 4.26, total protein 22.9, fat 5.5, fiber 1.6 and phosphorus 0.38 per cent, according to figures furnished by the manufacturer. The rats on the dog biscuits ate, including the milk, about 11 grams of food per day or approximately 14 per cent of protein (Nx6.25). Thus the crude protein supplied the rats on the two diets was equal in quantity.

Inasmuch as the quantity of protein provided in the experimental meat and the stock rations was above the critical level as defined by Simmonds (9), it was judged that the growth, reproduction and lactation responses would be partial measures of the quality of the meat protein providing, of course, that the accessory factors of the diet were furnished in optimal or ample proportions. If the quality of the meat provided was deficient, the growth, fertility and infant mortality results would be direct reflections of this deficiency.

## Results with Discussion

The litters of the parental generation on the basal diet grew at an excellent rate and flourished. The baby rats were of good weight when weaned, ranging from 40 grams to 62 grams in weight at 27 or 28 days of age. They were solid in body, frisky and playful, of good disposition and seemed in every way to be normal healthy rats. Their fur was thick, even, and of good texture. When the litters of the succeeding generations were weaned successfully they, too, made excellent growth. The mother rats of the first generation were not so successful in lactation as those of the parental generation. They gave birth to vigorous, healthy young, then failed to rear them. The first litters were born to the mothers of generation I when the mothers were approximately 12 weeks of age. The original plan was to continue not less than two females from each generation on the basal diet until each had produced at least five litters. Because the disasters in rearing the young were so numerous this plan was not carried out.

* Champion Dog Biscuit—Champion Animal Food Co., Minneapolis, Minnesota.

The disasters encountered in the rearing of the young were of two types, cannibalism and evident failure of the milk supply. When cannibalism occurred, it occurred as a rule soon after the birth of a litter. Where the failure of the milk supply was at fault, the mother rat neglected her young and left them to die without eating them. The young of such mothers had no milk in their stomachs.

An histological examination of the mammary gland tissues of these mothers revealed under-developed alveoli. This same observation was made by Watson (1). Evidently the dietary constituent or constituents directly responsible for the necessary mammary gland development were not sufficiently provided in our meat ration.

It was apparent that the basal diet though successful for growth and reproduction failed in many instances to meet the needs of lactation. Reproduction occurred at frequent intervals but the generations were carried on with difficulty because the mothers failed so repeatedly to rear their young. Watson (1) had found the growth of the rats retarded and sterility induced as the results of his feeding tests with an even more inadequate ox flesh diet. Our problem, then, seemed to be one of providing a more abundant source of the dietary constituent directly responsible for successful lactation.

For this reason, it was decided to supplement the basal ration with sources of the vitamins which have been recognized as promoting the milk supply. Accordingly the rats in the colony were divided into four groups, so distributed that rats from each of the generations were included in the several groups. The rations of the groups were as follows: Group I, meat for a control group; Group II, 5 per cent of yeast added replacing five per cent of the cornstarch and making a total of ten per cent of yeast; Group III, the salt mixture raised from four to five per cent; Group IV, the meat of the diet doubled. This was done by drying out the moisture of the meat until the weight was one-half that of the original and then mixing as before.

Two things were observed following these changes in the diet. Fertility noticeably decreased and many deaths occurred. On autopsy, hair balls were found in the stomachs or lodged in the intestines with hemorrhagic areas surrounding them. As the hair balls occurred in the rats from all the groups they can not be attributed to any one modification of the basal diet. Evidently they were formed while the rats were on the original basal diet and not after the dietary modifications were adopted.

Hair balls were observed by Mitchell, Bradshaw and Carlson (14)

to occur in the stomachs of 90 per cent of their rats fed the standard casein paste diet of Osborne and Mendel. They were not formed in the rats fed mixed foods, dry crumbly diets or liquid diets. The hair-ball formation was attributed by them to the high fat content and sticky consistency of the paste diet.

Later in the same year, Pappenheimer and Larimore (15) in a discussion of the incidence of the lesions which they found occurring in rats on artificial and deficient diets, speak of finding hair embedded in the ulcers and surrounded by an inflammatory region. They did not find the well-formed hair balls reported by Mitchell and her associates. The rats in which Pappenheimer and Larimore found the ingested hair were those on their diet 84, a dry diet, hence they do not agree with Mitchell, Bradshaw and Carlson that it was the fat of the diet that was at fault. They were not able, however, to analyze the factors in terms of known dietary deficiencies but felt that some other factor was involved.

Mitchell, Bradshaw and Carlson (14) observed that the females in which hair-balls were subsequently found had shown unusually poor reproduction and that if they bore more than one litter the number and survival of the later litters was poorer. This they thought indicated a progressive deterioration related to the formation and irritation of hair-balls.

It was our experience that the older rats were found to have, in almost every case, one or more well-formed hair-balls in their stomachs. Autopsies with microscopic examination of the internal organs were made following death, natural or otherwise. Sometimes the hair-balls were found well along in the intestines and surrounded by a well-defined hemorrhagic area. The occurrence of the hair-balls was regarded as an index of a defect in the basal ration employed, but since it contained but 12 per cent of fat in contrast to 25 to 27 per cent of fat as found in the Mitchell *et al* paste diet, it hardly seemed reasonable that they could be due to the fat content or stickiness of the ration. In this connection it is of interest to note that after the modifications of the basal diet designed to improve lactation were made, an account of which is given in a succeeding paper (16), there were no hair-balls found in any of the rats. This observation would seem to relate hair-ball formation to defective diet rather than to the fat content of the diet as the dietary modifications made did not, in any instance, affect the fat content.

Another observation of interest was that early senility appeared in the mature rats. The symptoms of senility are recorded by Simmonds (9).

The rats became unkempt in appearance. Their hair was uneven and thin. A yellow deposit resembling dandruff appeared on their backs. The skin on the ears, feet and tail became harsh and dry and the tails showed desquamative changes. Perhaps the senility changes, particularly as they are reflected in exterior conditions of hair and skin, are in part a result of impaired nutrition due to the presence of and the irritation produced by the hair-balls in the stomach. If so, the loose hair may be more readily swallowed during the rats' usual toilet preparations and thus there is a vicious circle operating to make matters worse, once a hair-ball formation is started. At any rate, one wonders why no hair-balls were observed after the diet was improved for lactation, as the improved diet was eaten during lactation periods only.

The rats on the control ration, that is, the dog biscuit ration as previously described, reproduced and reared their young successfully and without evidence of disasters through the three generations.

## Summary and Conclusion

Excellent growth and reproduction were secured in rats consuming a meat ration supposedly well fortified with the necessary vitamins and mineral matter and having lean muscle beef as the main protein constituent. Disasters in lactation, *i.e.*, cannibalism and failure of the milk supply, were numerous among the rats fed the meat ration, whereas no difficulties were encountered with the rats fed the control ration.

Modification of the experimental meat ration by increasing its yeast content, increasing the salt mixture and doubling the protein content, resulted in no appreciable improvement in the results.

Numerous hair-balls were found in the stomachs and intestines of the adult rats on the meat ration and were frequently the cause of death. Early senility was evident.

The meat ration proved suitable for growth and reproduction but failed to meet the needs of the mother rats during the critical period of lactation.

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# MEAT IN NUTRITION

## II. SOME DIETARY FACTORS INFLUENCING LACTATION*†

*By*

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Received for Publication—March 28, 1930

### Introduction

INVESTIGATORS in biology and nutrition agree that the period of lactation is a critical time in the life of a mother. Since this function requires a reserve of energy which is dependent upon the maintenance of optimum nutrition, the ability to wean vigorous, healthy progeny has come to be regarded as an index of the adequacy of the mother's diet at this time. In a previous report (23) attention was called to the fact that the meat diet used, which proved adequate for growth and reproduction in albino rats, must be supplemented during lactation if optimum and consistent results were to be secured. The present investigation reports the results obtained when dietary supplements of various kinds were added to the basal diet during the latter part of pregnancy and throughout the period of lactation.

### Review of Literature

In a consideration of the relation of diet to lactation attention is usually given to the requirements in protein, minerals and vitamins. Of the proteins used in nutrition experiments, casein has been the one most frequently selected for the basal protein because of its ease of handling and availability. However, some investigators have used meat. Meat is an important constituent of the average human diet and one whose rôle in nutrition has been often disputed. In 1902, Watson (36) after studying the influence of a liberal meat diet on the fertility and lactation ability of several classes of women in England, followed by a series of control experiments with rats, decided that the increased meat consumption probably had a bearing on the failure of mothers, both human and animal, to nurse their young.

More recent investigators, however, have concluded from animal feeding experiments that meat-fed mothers produce litters showing improved weight and growth. Hitchcock (11) found that the young from meat-fed rats grew more rapidly and at thirty days weighed about 20 per cent more than the controls. He fed the mother rats daily 8 to 10 gms. of fresh lean meat cooked rare, in addition to what he considered an adequate diet. McCollum, Simmonds, and Parsons (16) fed varying percentages of kidney, liver, and muscle meat to rats. The group receiving 20 per cent of the kidney and liver proteins reproduced successfully through four generations.

* The data presented are taken from the dissertation submitted by Louise Jenison Peet to the Graduate School of Iowa State College in June, 1929, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

† Published with the permission of the Director of the Iowa Agricultural Experiment Station.

MacLeod (15) stated that rats receiving 17.5 per cent of protein from fresh lean beef showed improved reproduction and lactation but the animals in the second generation grew subnormally.

Slonaker (35) found that the length of life and weight of albino rats increased when animal protein from table scraps was added to the diet. He considered that the animal protein was either more easily utilized or acted as a stimulant on all the body cells, so that the other food was used more completely.

The advantages of a liberal protein intake during lactation are frequently stressed. McCollum and Simmonds (16) found that there was no evidence of toxicity even when the diet contained the equivalent of 70 per cent of protein from muscle, kidney, or liver tissue; and Hartwell (10) suggested that lactation may require supplementary protein.

Of the vitamin needs for lactation, special emphasis has been placed on Vitamin B. Since Vitamin B* stimulates growth by increasing the assimilation and utilization of the food (13) it may easily follow that all classes of food stuffs should be balanced with vitamin B (28). Hartwell (9) stated that this vitamin seemed connected with protein metabolism, and Nelson (22), experimenting with rats on a dried whole milk diet, to which varying percentages of casein were added, showed that lactating rats on such high protein diets needed an increased amount of vitamin B in order to wean vigorous healthy young.

According to Reader and Drummond (29) normal growth in rats resulted only when the ratio of protein to yeast was five or under. Sherman and Gloy (31), however, found that increased protein did not call for a corresponding addition of vitamin B for the maintenance of the rat at the age studied. Animals apparently "do not possess any large available reserve" of vitamin B (26), and the maternal organism seemingly will not sacrifice what it has for the young. (18, 21). Experiments with rats have shown, therefore, that a much greater amount of this vitamin is necessary for rearing the offspring than for growth (24). Different investigators estimate that there should be added to the lactation diet as a source of vitamin B from three to five times the amount of yeast needed for maintenance (4, 9, 17, 21). Moore (21) fed rats a synthetic diet in which 18 per cent of casein was the source of protein. After increasing the amount of yeast in the diet from 2 to 7 per cent, he concluded that even 7 per cent was apparently near the minimum requirement.

Vitamin B is now known to consist of at least two factors. Chick and Roscoe (2), Goldberger (7), Evans (6), Sherman (32), Levene (14), and others have studied its dual nature. Following the report of the special committee appointed to make recommendations on nomenclature of vitamins by the American Society of Biological Chemists (30), the two factors of vitamin B have been designated by the letters B and G. Both factors are necessary for growth (35), but B is especially concerned with the prevention of polyneuritis, and G with the promotion of growth "by making possible a more normal nutritional condition" (12). It is believed that natural foods used as sources of vitamin B contain both factors (2), but experiments have proved that they are not always present in equal amounts (6, 33).

In controlled experiments with rats autoclaved yeast is frequently used to furnish the G factor of vitamin B (12, 32), and Evans (6) found tikitiki, the alcoholic extract of Philippine rice polish, a satisfactory source of the antineuritic element. He concluded (4), that the need for the addition of yeast during lactation was due solely to its antineuritic factor, since normal lactation occurred in rats when he did not increase the amount of yeast but supplemented it with tikitiki.

The findings of Evans (5) and those of Harden and Silva (8), would indicate that rats are not susceptible to scurvy and hence may not require the antiscorbutic vitamin in their diet, although some vitamin C is found in the rat's liver. However, Harden and Silva (8) and Manville (8) observed that when lemon juice was added to the drinking water the rats showed better growth than when the lemon juice was omitted. Drummond and Crowden (3) also included 5 per cent

* Vitamin B, as used in this paper, refers to the Vitamin B complex.

of lemon juice in their normal and high protein diets, which contained 20 per cent and 83 per cent of caseinogen respectively.

Lemon juice, like orange juice, contains vitamin B and some vitamin A, hence part of the effects noted may have been due to the increase in these vitamins. Sherman and Gloy (31) fed 4 cc. of orange juice, as a sole source of vitamin B, to rats on different levels of protein intake and found it entirely adequate.

It is now commonly accepted that there is a specific vitamin essential for reproduction, vitamin E. Evans and Burr (5) observed that the young of rats on a synthetic diet containing 32 per cent of casein, even when given an increased percentage of yeast during lactation, developed a form of paralysis in the hind limbs at about the nineteenth or twentieth day of suckling. They experimented with various factors and concluded that the paralysis was caused by a lack of vitamin E. About the fifteenth day after parturition seemed to be a critical period in the development of the neuro-muscular system of the offspring and if, beginning at this time, five drops of ether-extracted wheat germ oil were fed daily, the litter was weaned in a normal condition. They also found that these animals receiving the wheat germ oil were heavier than the litter mate controls.

In a diet designed to insure rapid growth in albino rats Mendel and Cannon (19) included a "liberal" amount of wheat germ to provide vitamin E. The protein was furnished by 12 per cent of meat scraps and 12 per cent of casein (27). Miller (20) found that no additional yeast furnishing vitamin B was needed during lactation if the diet contained 10 per cent of autoclaved wheat embryo.*

Such minerals as are needful for the building of the body tissues and fluids must be included, not only for the development of the young, but for the proper maintenance of the mother's vitality against the drain which lactation makes upon it. An inadequate inorganic salt intake may seriously affect the nursling, particularly in the later stages of lactation when the maternal reserve has become depleted.

## Experimental

It is obvious that in these various studies verification of the nutritive needs of lactating animals is difficult because the conditions of the experiments are not comparable. It seemed, therefore, worth while to feed groups of rats having a common basal diet the modifications proposed by the other investigators to determine if possible which would be the most successful in overcoming the difficulties encountered in lactation. In addition, as a control, a group of rats was kept on the basal meat diet without any supplementary constituent, and a second control group was fed the modified Steenbock diet† which is used in this laboratory for the rearing of the stock animals.

The following modifications of the basal diet were tried:

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* Clayton, whose report appeared after this paper was submitted for publication (This Journal, II, 491; III, 23), found that 3 per cent of wheat germ oil added to the basal muscle beef ration improved lactation in a few cases. When the fat, lard, was omitted and 2 per cent of cod liver oil used. lactation was improved also.

† Modified by adding to the original diet 2 per cent of yeast and 10 per cent of wheat germ, and using 64 parts of yellow cornmeal in place of 76. Steenbock Stock Ration. Science 58:449. 1923.

A. Increasing the yeast from 5 to 15 per cent, or 3 times the amount used for maintenance.

B. Addition of autoclaved yeast to supply the G factor of vitamin B.

C. Addition of tikitiki to supply the antineuritic factor of vitamin B.

D. Using lemon juice to provide vitamin C.

E. Using ether-extracted wheat germ oil to supply vitamin E.

F. Increasing the amount of meat protein.

G. Increasing the amount of meat protein and increasing the yeast to 15 per cent.

*Criteria for Evaluating Results.* As a basis for determining which diets were most successful in promoting lactation, the following criteria were considered: 1. The age of the mothers when the first litter to be successfully raised was born; 2. The gain or loss in weight of the mothers during the lactation period; 3. The number of young successfully weaned by the mothers on a given diet; 4. The weight and physiological condition of the young at time of weaning.

## Experimental Procedure

This study, using albino rats as the experimental animals, has been carried through three generations after the parent generation with which the experiment started. The parent generation had not been previously fed a meat diet. It was bred, therefore, only until groups on the various diets were obtained for a first generation. No litter was retained to be used for the first generation until the parent rats had been fed the meat diet for at least four weeks before the young were born.

In the first generation there were nine groups of six rats each on the basal meat diet, four females and two males in each group. As the females became pregnant they were transferred to individual cages and the supplementary food factors added to their basal meat diet.

Since it is usually impossible to diagnose a dietary lack in the nursing young until too late, it seemed better to feed the mothers the additional constituents during the latter part of the period of pregnancy as well as throughout lactation. In each case the group in the first generation was fed the same supplementary diet during pregnancy and lactation as the parents had received. This same method was followed in each succeeding generation so that each supplementary factor was carried through one family group from generation to generation.

A few days after birth the litters were reduced to six animals each, and on the twenty-first or twenty-second day they were weaned. Usually

two or three mothers in a group had offspring at approximately the same time. Hence, the group of rats constituting the second generation was selected from more than one litter. This avoided too great inbreeding.

When the six animals each had been selected to make up the various groups for the second generation, the first generation groups were reduced from four females and two males each, to two females and one male. Each female was then remated until she had borne five litters. This same procedure was followed in the groups for each succeeding generation.

The experimental diets and tap water were fed *ad libitum*. A record of the food consumed each day was kept. When there was more than one animal in a cage this record represents the average food consumption. The rats were weighed at least once a week. The composition of the basal diet used was as follows: meat, pressure cooked lean round of beef 50; cornstarch 28; dried brewery yeast 5; salt mixture (25) 4; butter fat 8; cod liver oil 2; agar 2; NaCl 1.

The butterfat used was melted over hot water and separated from curd, water and salt. Agar was included for roughage. Squibbs cod liver oil was used. The meat was ground and canned by processing at 15 to 16 pounds pressure for one hour and fifteen minutes. Fifty pounds of meat were processed at one time and used as needed. The meat furnished about 14 per cent of protein. The yeast used was dried brewery yeast obtained from the Northwestern Yeast Company.

Of the nine groups of animals, two groups served as controls; one was retained upon the basal diet without supplementary feeding during lactation, and another was fed the modified Steenbock diet, supplemented with whole fresh milk *ad libitum*. The seven other groups were fed the basal diet with the following specific additions to the diet during pregnancy and lactation; Group A, 10 per cent of yeast, substituted for an equal amount of the cornstarch; Group B, 6.5 per cent of autoclaved yeast, substituted for cornstarch; Group C, 4 drops of tikitiki,* fed separately each day; Group D, 12 per cent of lemon juice in the drinking water; Group E, 5 drops of ether extracted wheat germ oil fed separately daily; Group F, twice the amount of meat; Group G, twice the amount of meat with 15 per cent of yeast.

Environmental conditions, such as temperature, humidity, light and

* Tikitiki, an alcoholic concentrate of the antineuritic factor of vitamin B (Phil. Jour. Sci. 19:67-73. 1921) is made by the Philippine Bureau of Science for distribution to the natives who are afflicted with beriberi. We are greatly indebted to Dr. A. H. Wells, formerly chemist in charge, for the supply which has been used in these experiments.

sanitation, were as constant as could be maintained in the nutrition laboratory.

The albino rats were kept in individual all metal cages with false bottoms, thus preventing access to the feces. During the growing and mating periods the rats were kept in large metal cages 9″×12″×18″. When the females became pregnant they were transferred to individual cages.

At autopsy a microscopic examination for intestinal parasites in representative rats on the various diets was made. The rats showed unusual freedom from worms, amoebae and trichinae, thus attesting to the adequacy of the means taken to insure the most advantageous environment possible.

TABLE I

AGE OF MOTHERS AT BIRTH OF FIRST LITTERS RAISED AND THEIR GAIN OR LOSS IN WEIGHT DURING THE LACTATION PERIOD

| Diet | Average age of mothers at birth of first litter raised (days) | Average gain in weight of mothers during lactation (gms.) | Average loss in weight of mothers during lactation (gms.) |
|---|---|---|---|
| Basal meat | 211.6 | — | 28.2 |
| A<br>15% yeast | 127.8 | 7.1 | — |
| B<br>Autoclaved yeast | 113.4 | 8.3 | — |
| C<br>4 drops tikitiki | 137.5 | — | 16.5 |
| D<br>12% lemon juice | 109.4 | — | 5.5 |
| E<br>5 drops wheat germ oil | 109.8 | — | 21.2 |
| F<br>Increased meat | 114.8 | — | 6.3 |
| G<br>Increased meat and 15% yeast | 119.0 | — | 3.2 |
| Steenbock stock (control) | 132.0 | 3.0 | — |

## Discussion of Results

Protocols of animals from each group through the three generations are given in Tables I and II. The records for the parent generation are not included because, as has been stated, these rats had been on the meat diet for a few weeks only and their previous history was unknown.

It was assumed that one measure of the adequacy of the different diets for lactation would be the age at which the mother rats were first able to raise their litters successfully. Rats normally become pregnant for the first time at about 90 days of age. Allowing for a gestation period of from 21 to 23 days the young would be born when the mother rats were between

Table II

Percentage of Young Reared in the Three Generations and Their Weights at 21 Days

| Diets | Mother rats (no.) | Litters born (no.) | Litters reared (no.) | Young reared on each diet (per cent) | Average weight of young at 21 days (gm.) |
|---|---|---|---|---|---|
| Basal meat | 5 | 21 | 5 | 24.7 | 34.9 |
| A 15% yeast | 10 | 44 | 19 | 42.4 | 37.3 |
| B Autoclaved yeast | 7 | 27 | 17 | 62.5 | 33.2 |
| C 4 drops tikitiki | 8 | 22 | 11 | 32.0 | 32.0 |
| D 12% lemon juice | 9 | 27 | 13 | 53.3 | 34.5 |
| E 5 drops wheat germ oil | 7 | 26 | 13 | 62.2 | 33.3 |
| F Increased meat | 8 | 25 | 14 | 57.1 | 37.3 |
| G Increased meat+15% yeast | 8 | 26 | 16 | 71.9 | 42.3 |
| Steenbock stock (control) | 9 | 19 | 10 | 66.6 | 34.2 |

110 and 120 days old. This was found to be true with slight variations on all the diets except the basal meat. (Table. I.) In this case the average age of the mothers at the birth of the first litters raised was 211.6 days.

A further criterion for determining which diets were most successful in promoting lactation was whether the mothers gained or lost in weight during the nursing period. It is generally agreed that a diet is adequate for lactation if the mother's weight remains about constant during this period, or shows only slight gain or loss. Although the average losses in weight of the mothers on the diets to which 4 drops of tikitiki were added and to which 5 drops of wheat germ oil were added, might be considered greater than the amount thought desirable, it was here again that the mothers on the basal meat diet showed the greatest loss. (Table I). Their average loss was 28.2 gm. These findings confirm the results of the preliminary experiment which indicated that the basal meat diet was inadequate for lactation (23).

In evaluating the diets for the effects upon the offspring, the percentage of young reared and their average weights at 21 days will be considered. These results are recorded in Table II. It will be seen that on the basal diet a very few litters were raised. Of the 93 young born to mothers in generation I, 72 per cent died or were eaten within two or three days after birth. The rest were so emaciated that they were discarded. In only three cases was there any indication of lactation. Two litters have been raised in generation II, but in generation III no litters have been born, although the four females on the diet are each approximately 160 days old.

The autopsy of the females in generation I revealed a great amount of fat surrounding the kidneys and intestines and filling the abdomen. Most of the rats reared on the meat diet with the modifications, in contrast to those reared on the basal meat diet alone, were found to have a moderate or even a small amount of body fat. It is a common observation that when the food consumed is metabolized by the animal or the human to form body fat, lactation is impaired and the offspring do not receive the nourishment they require.

The record for the 3 generations of mothers fed ration A, containing 15 per cent of yeast as a source of vitamin B, shows a rather low percentage of young reared, only 42.4 per cent. Whether or not yeast is a wholly satisfactory source of vitamin B is apparently open to question. It may be that other constitutents of the yeast when present in the increased quantity have a detrimental effect.

Some of the most favorable results in any of the groups were obtained

from the mother rats receiving autoclaved yeast. In the first generation, 95.1 per cent of the offspring was weaned. This confirms the observations of Sherman and Sandels (34) that the G factor of vitamin B "may be expected to play a significant part in inducing a better-than-average nutritive condition." The results obtained in the second and third generations were less favorable. This may be due to individual differences in the rats.

It is to be noted that in the first generation several of the offspring had ringed tails with a tendency for part of the tail to drop off. In one litter one of the young never fully developed and weighed only 13 gm. at at the time of weaning. The young in the second and third generations showed no abnormalities.

Burr and Burr (1) write of a caudal necrosis in the rat apparently caused by a deficiency of fat in the diet. Since the diets used in the present investigation were well supplied with butter fat and cod liver oil, lack of fat cannot be the cause in this case.

Tikitiki was the supplementary factor in diet C. Only 32 per cent of the young have been raised. After bearing three litters each, the mothers of generation II showed no sign of further pregnancies, although observations were made for a period of more than three months. They had a tendency to store large amounts of body fat. The rats in generation III have not reared any of their litters. These results fail to sustain the observations of Evans and Burr (4) that tikitiki without increase of yeast produces normal lactation.

The young of one litter on the tikitiki diet showed ringed tails. Two of the young had also a meager amount of hair on the hind quarters. Goldberger (7) noted that rats fed on an extract containing the antineuritic factor of vitamin B developed a pellagra-like condition, manifesting itself in affections of the skin and loss of hair.

Similar conditions were found in some cases on the diet containing the 5 drops of wheat germ oil. In the second generation runts appeared in two different litters, and the young had a moth-eaten appearance. One was almost bald. A larger amount of wheat germ oil might prove more efficacious.

Diet F contained a double portion of meat. This increase in protein seemed to have a favorable effect, for although only 57.1 per cent of the young have been reared, this low percentage is due almost wholly to failures in generation I. In generations II and III only two litters out of 12 failed to grow to maturity. It is to be noted that in the second genera-

tion the more favorable results were obtained with litters born to older mothers. With a more liberal inclusion of protein in the diet there was a delay in the onset of pregnancy. Moreover after bearing one or two litters the mothers became sterile. In both generations I and II, many of the young had ringed tails and the ends dropped off, giving a bob-tailed third generation.

Corresponding results were obtained with the inclusion of 15 per cent of yeast in the increased meat diet. In generation I the mothers were four and one-half to five and one-half months old at the birth of their first litters. About 75 per cent of the young were reared in the first and second generations. The third generation was less successful, resorptions apparently occurring in several cases.

When the average weights of the young at 21 days are compared, the offspring of rats on the diets containing increased protein show greatest weight. Since a favorable percentage of young were reared on these diets, it may be concluded that increased protein is an aid in obtaining successful lactation. Better results might have been obtained on the other diets had the amount of meat been increased. A further experiment is now being carried on in which the diet contains increased meat plus autoclaved yeast. The results obtained so far would seem to indicate optimum lactation and growth.

## Summary and Conclusions

Using as the basal diet a well balanced, modified, synthetic ration, containing pressure cooked lean beef round as the chief source of protein, reproduction and lactation responses in albino rats were observed through three generations. During the latter part of pregnancy and throughout lactation there were added to the basal diet those dietary modifications which have been recommended from time to time as being specifically connected with milk production. Past experience with this basal diet had shown that it enabled the rats to respond normally except during the critical period of lactation.

The conclusions are:

1. All the diets, except the basal, apparently enabled the mothers to bear litters at the normal age of about three months. In some individual cases, however, increasing the meat protein of the diet, seemed to delay the onset of pregnancy.
2. The diets modified by the additions of tikitiki and wheat germ oil,

and the control basal diet were least efficient in maintaining the weight of the mothers during lactation.

3. Mothers on the increased meat diet plus 15 per cent yeast weaned the largest percentage of the young born in the three generations. But in the first and second generations an even larger percentage was reared by the mothers fed the basal diet with the autoclaved yeast addition.

4. The growth response of the young on the different dietary modifications was greatest for diet G, increased meat protein plus 15 per cent yeast.

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# MEAT IN NUTRITION

## III. HEMOGLOBIN FORMATION*†

*By*

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Received for Publication—March 28, 1930

### INTRODUCTION

THE experimental work reported in paper II was an attempt to discover what constituents should be added to the basal meat diet used to obtain successful lactation. This diet had been found to be adequate for reproduction and growth, but on it the mother rats were often unable to nurse their offspring (6).

Further confirmation of the adequacy of a ration may be obtained perhaps by a determination of the percentage concentration of the hemoglobin of the rats on the ration. Accordingly, the number of gm. of hemoglobin per 100 cc. of blood was determined for the young of the rats on the different diets used in the experiments reported in paper II (6).

### REVIEW OF LITERATURE

Comparatively few experiments have been carried on for the determination of the relation between the composition of the diet and hemoglobin formation in the rat. Extensive studies by Minot and Murphy (5), Hart and Steenbock, *et al.* (3), and Robscheit-Robbins and her associates (7), have been made which demonstrate the relation of diet to hemoglobin building in dogs, rabbits and man.

Cartland and Koch (1), however, have studied the relation between certain proteins and vitamins in the diet and the hemoglobin forming process in young growing rats. They fed various synthetic diets, using as the source of protein 10 per cent of wheat gluten and casein, and 15 per cent of hemoglobin combined with tryptophane. They found that wheat gluten and casein were about equally efficient for promoting hemoglobin synthesis in the rat and that no more satisfactory results were obtained with hemoglobin and tryptophane. They further concluded, after "using the severe anemia type of experiment," that the blood forming process in the rat was not dependent upon the presence of vitamins A, B, or E in the diet. After being bled their rats seemed to regenerate hemoglobin at a normal rate when sources of vitamins A, B and E had been omitted from the diet and the later addition of these vitamins to the diet was without effect upon the hemoglobin regeneration.

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* The data presented are taken from the dissertation submitted by Louise Jenison Peet to the Graduate School of Iowa State College in June, 1929, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Cartland and Koch (1) not only estimated the percentage of hemoglobin and made red blood counts, but also determined the blood volume. Waddell, Steenbock, and associates (10) in their studies on anemia in rabbits decided that the hemoglobin determinations gave sufficient information for the purpose desired and that the red blood counts added so little to these results that they did not justify the time and technic required. In an earlier study of nutritional anemia, Hart, Steenbock, Elvehjem and Waddell (3) had found that diets supposedly furnishing vitamin E were similar to diets efficacious in curing the anemia. Some diets said to be lacking in vitamin E and hence not effective for reproduction might really be causing anemia in rats and consequently abnormal reproduction.

Williamson and Ets (11) found that the percentage of hemoglobin varied with the age of the rats. For their study they used normal rats on a standard casein diet. The hemoglobin concentration fell steadily from birth to the fiftieth day of life, then gradually rose to a maximum on the one hundred and fiftieth day. They assumed that the explanation might be due to the fact that rats, like other animals, contain a much higher percentage of iron in their livers at birth than later in life. Milk is noteworthily low in its content of iron. Therefore, unless a rat at birth contained a sufficient reserve of iron to carry over the suckling period, this store of iron would become partially depleted and a temporary anemic condition would result. After the rats had begun to eat for themselves and to obtain iron from their food the anemia would gradually disappear.

Sure (9) and his associates obtained somewhat different values for the variation of the hemoglobin content of the blood of albino rats with age. They found a decrease in hemoglobin from the third to the fifteenth day of lactation. From the fifteenth day until weaning the hemoglobin content remained about stationary, but rapidly increased in amount during the following five days. Comparable results were obtained by Smythe and Miller (8).

## Experimental

Hemoglobin percentages should be comparable if obtained from young rats at approximately the age of weaning, and after their mothers had been for 4 or 5 weeks on the specific diets. Accordingly, all the hemoglobin concentrations were measured when the litters were between 21 and 30 days of age. Two separate hemoglobin determinations were made on each rat. The method of Cohen and Smith (2) was followed.

The rats were bled under ether anesthesia by cutting off the ends of their tails. By means of a calibrated pipette 25 c.mm. of the blood were measured into 2475 c.mm. of the 0.1 N hydrochloric acid. The blood pipette was rinsed out by drawing acid solution into it several times.

Readings were made with the Leitz Burker hemoglobinometer which has a "strictly symmetrically disposed system of rays. This arrangement entirely eliminates the effect of the solvent."

## Discussion of Results

The results of the hemoglobin determinations are recorded in Table I. The mean values for the gm. of hemoglobin per 100 cc. of blood, for the rats on this experiment, are somewhat lower than those given by Williamson and Ets (11). They seem moreover, to be slightly higher in value than

those obtained for rats between 20 and 30 days of age by Sure, Kik, and Walker (9).

When the mean value of the hemoglobin concentration of the blood of rats on the autoclaved yeast was compared with the mean values of the hemoglobin concentration of the blood of rats on the other diets, the mean differences were found to be significant, in favor of the autoclaved yeast diet, only in the case of rats on the increased meat and increased meat plus 15 per cent yeast rations. In these two cases the mean differences were found to be more than three times the standard deviations. Similar results were obtained in comparing each of the other diets with the increased meat diet and the increased meat plus the 15 per cent yeast. In each case the mean differences were found to be more than three times the standard deviation and hence significant. It appears, therefore, that diet by affecting the milk of the mother has a measurable effect upon the hemoglobin concentration of the offspring.

## Summary

Duplicate determinations were made of the hemoglobin concentration of the blood of 82 young rats selected at random from the second and third generations of albino rats fed a modified synthetic diet in which lean muscle beef was the chief source of protein. To this ration during the suckling period were added certain modifications recommended as valuable for promoting successful lactation.

The diets which contained, during the suckling period, an increased percentage of yeast, autoclaved yeast, tikitiki, lemon juice, or wheat germ oil, led to significantly greater hemoglobin formation in the young than the diets containing increased protein.

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TABLE I

HEMOGLOBIN CONCENTRATION OF BLOOD OF YOUNG OF ALBINO RATS ON THE EXPERIMENTAL DIETS*

| Mother (No.) | Supplemental diet | Age of young (days) | Young tested (No.) | Hemoglobin grams per 100 cc. blood | | Mean value |
|---|---|---|---|---|---|---|
| | | | | Determination I (grams) | Determination II (grams) | |
| II 1656 | A (15% yeast) | 30 | 2 | 10.63<br>11.16 | 10.67<br>11.16 | 10.91±0.13 |
| I 1397 | A | 21 | 5 | 10.13<br>9.98<br>9.69<br>10.09<br>10.02 | 10.17<br>9.90<br>9.63<br>10.10<br>9.90 | 9.96±0.05 |
| I 1461 | B (autoclaved yeast) | 25 | 5 | 10.70<br>10.82<br>10.63<br>10.93<br>11.07 | 10.72<br>10.90<br>10.17<br>10.90<br>10.88 | 10.77±0.07 |
| I 1660 | C (tikitiki 4 drops) | 29 | 5 | 11.21<br>11.00<br>10.97<br>10.98<br>10.29 | 10.61<br>10.84<br>10.85<br>11.08<br>10.40 | 10.82±0.09 |
| I 1662 | C | 21 | 5 | 10.60<br>10.77<br>10.54<br>10.55<br>11.11 | 10.75<br>10.64<br>10.68<br>10.45<br>11.02 | 10.71±0.07 |
| II 1752 | D (12% lemon in drinking water) | 30 | 5 | 12.79<br>12.91<br>12.98<br>12.89<br>12.77 | 13.01<br>12.96<br>13.43<br>12.79<br>12.86 | 12.94±0.06 |
| II 1753 | D | 25 | 3 | 10.68<br>11.47<br>11.49<br>11.49<br>11.27 | 10.80<br>11.55<br>11.38<br>11.57<br>11.40 | 11.30±0.10 |

* (With the exception of the basal diet on which no young were reared)

TABLE I (*Continued*)

| Mother (No.) | Supplemental diet | Age of young (days) | Young tested (No.) | Hemoglobin grams per 100 cc. blood | | Mean value |
|---|---|---|---|---|---|---|
| | | | | Determination I (grams) | Determination II (grams) | |
| I 1489 | E (wheat germ oil 5 drops daily) | 28 | 5 | 9.96<br>10.39<br>10.21<br>10.02<br>9.94 | 10.07<br>10.01<br>9.90<br>9.84<br>9.48 | 9.98±0.07 |
| II 1759 | E | 21 | 5 | 10.80<br>10.78<br>10.83<br>10.83<br>10.83 | 10.86<br>10.90<br>10.88<br>10.97<br>10.83 | 10.85±0.07 |
| II 1844 | F (50% added meat) | 22 | 5 | 8.42<br>9.29<br>9.66<br>9.62<br>8.78 | 8.62<br>9.81<br>9.93<br>9.47<br>8.86 | 9.24±0.18 |
| II 1846 | F | 21 | 5 | 9.61<br>9.77<br>9.56<br>9.73<br>9.38 | 9.61<br>9.61<br>9.53<br>9.80<br>9.06 | 9.57±0.06 |
| II 1847 | F | 22 | 5 | 9.30<br>9.15<br>9.19<br>8.45<br>8.19 | <br>9.48<br>9.53<br>8.43<br>7.93 | 8.85±0.18 |
| I 1498 | G (15% yeast 50% protein added | 21 | 5 | 8.30<br>8.57<br>8.39<br>7.85<br>8.28 | 8.25<br>8.35<br>8.34<br>7.93<br>8.38 | 8.26±0.06 |
| I 1499 | G | 21 | 5 | 8.56<br>8.43<br>8.43<br>8.38<br>8.38 | 8.45<br>8.39<br>8.52<br>8.52<br>8.70 | 8.48±0.09 |

| Mother (No.) | Supplemental diet | Age of young (days) | Young tested (No.) | Hemoglobin grams per 100 cc. blood | | Mean value |
|---|---|---|---|---|---|---|
| | | | | Determination I (grams) | Determination II (grams) | |
| I 1556 | Steenbock Stock | 27 | 5 | 9.94 | 10.05 | |
| | | | | 9.77 | 9.79 | |
| | | | | 9.74 | | 9.98±0.08 |
| | | | | 9.92 | 9.87 | |
| | | | | 10.14 | 10.57 | |
| II 1804 | Steenbock Stock | 22 | 5 | 9.46 | 9.24 | |
| | | | | 9.21 | 9.56 | |
| | | | | 9.48 | 9.19 | 9.34±0.04 |
| | | | | 9.44 | 9.42 | |
| | | | | 9.23 | 9.19 | |
| II 1805 | Steenbock Stock | 22 | 5 | 8.35 | 8.46 | |
| | | | | 8.23 | 8.12 | |
| | | | | 8.33 | 8.00 | 8.34±0.06 |
| | | | | 8.67 | 8.41 | |
| | | | | 8.50 | 8.37 | |

# Editorial Review

## RECENT ADVANCES IN THE STUDY OF BASAL METABOLISM

### PART II

### *Basal Metabolism and Surface Area*

SURFACE area determinations have played an important part in all work on basal metabolism since the time when Rubner (1) and Richet promulgated their doctrine that among the warm-blooded animals metabolism is proportional to the surface area. Rubner emphasized the fact that the different animals should be studied under similar physiological conditions, a point that is sometimes neglected by those who discuss the so-called "surface area law." There is as yet no strong proof that the relationship between surface and metabolism is a causal one and most experimenters believe that the surface area furnishes the best index of protoplasmic mass which in turn has a causal relationship to the total heat production. There is as yet no proof of this protoplasmic mass theory. The discussion of the validity of the surface area law has been fairly active for many years, but has never deserved the term "polemic" ascribed to it by a recent reviewer (2). Perhaps the most encouraging work in the field of respiratory metabolism during the last three or four years has been the painstaking study of this subject. There is a marked revival of interest not only in accurate methods of determining the surface area of different animals but also in the systematic attempt to find truer basal values for the different species.

It is obvious that if we are to express our results for basal metabolism tests in terms of surface area, the error of the result will be just as great as the error in the surface area formula. The discussion is in part applicable to the generally adopted Harris-Benedict standards, since they are calculated from height and weight, the two factors that enter into most of the surface area formulas.

In 1915 and 1916 the Sage investigators put out two formulas for calculating the surface area, devised chiefly by Delafield Du Bois. The "linear formula" was based on the measurement of the average length and circumference of each part of the body. The "height-weight formula" was obtained by multiplying powers of the height and weight by a constant ($H^{0.725} \times W^{0.425} \times 71.84$). The classical formula of Meeh, weight raised to the $\frac{2}{3}$ power, gave good results with people of average shape but for

people of unusual shape better results were obtained if the height was taken into consideration. We must remember this in our review of all the tests of surface area formulas. If one wants to prove that a formula based on weight alone is the best, all one has to do is to take a series of subjects of different weights but approximately the same shape. The Sage investigators in their rather small series of subjects included a cretin, a marantic baby, a living skeleton and a woman so fat that she was called a "human pork barrel." They wanted a formula that could be used in the clinic.

For a good many years there was no checking of the accuracy of these formulas except in the Sage laboratory. Recently, however, a good many observers have been determining the surfaces of animals and of men and have calculated the errors in the formulas. Takahira (3), measuring the surfaces of ten Japanese men found the Sage height-weight formula averaged 0.9% too low, Wörner's work indicated that it was about 1.45% too high. Takahira found the best fit for his ten subjects was obtained with a formula only slightly different from the Sage height-weight method. He suggested using a K of 72.46.

Takeya (5) measured 22 Japanese by a slightly different method and concluded that the best formula to calculate the surface area was the Sage height-weight method with a K of 75.05. He noted that the constant seems to vary with the height in that when the height increases the K becomes smaller.

Stevenson (6) in a preliminary report states that the total area as estimated from the Sage linear formula gives remarkable agreement with the areas as actually measured by him on his Chinese men but that this is due to fortuitous compensation between errors in different regions. He suggests a new set of constants for the different parts of the body to be used with the Chinese. He is quoted as saying that the Sage height-weight formula gives higher results than the linear with the Chinese. MacLeod, Crofts and Benedict (7) found that it gave 3.0% lower results with Oriental women; Necheles (8) found the height-weight on the average 4.4% higher for the Chinese; Waddell, Jan and Ch'en (9) measuring 74 male Chinese students observed that the average variation between the two methods was plus or minus 1.25% which is about the same as is found among Europeans and Americans.

Actual measurements of surface area of women were relatively few in number until Mrs. Bradfield, (10, 11) working at the University of Missouri Agricultural Experiment Station, undertook the measurement of 47 young women. She employed a surface integrator described by Brody and

Elting (12). It consists of two wheels, a revolution counter, and a device for marking the area that has been covered. Knowing the surface area of the space between the two wheels, it is easy to roll the apparatus over the skin and in this fashion calculate the area that has been covered. Special calculations have to be made for hands, feet and head. This device is similar to one introduced by Bordier (13) and employed by Frontali (14) in his measurement of children. Mrs. Bradfield, in the one case tested, obtained excellent agreement with the mould method. Brody and Elting have in two cases noted close agreement between this integrator and the measurement of the hide of the slaughtered animal. Mrs. Bradfield found that the Sage height-weight formula averaged 1.8% higher than the areas as measured by the integrator (range −4.9 to+9.7 per cent). The Sage linear formula was 5.7% higher. The linear method of Wörner gave better results, showing a mean deviation of only 0.2%, though the range was about the same as for the Sage formulas. Mrs. Bradfield does not suggest a new formula for women, but does believe that if the Sage height-weight formula is used for them, a correction of 2.0% should be made.

The Sage formulas were not intended to be used with small children so we are not surprised when errors are found in the case of infants and fetuses. Frontali, using the integrator method, determined the surfaces of 33 living boys and girls from 24 days to 12 years of age. In all of the children under the age of 1 year, his results were lower than those calculated from the Sage height-weight formula, the average being −7.9%. All but five of the cases came within 10.0%, the greatest error being 18.9%. With children over 12 months of age the deviation of the formula with regard to sign was −1.6% and the largest divergence was +13.4%. The Lissauer formula with a K of 10.3 gave better results than the Sage formula. Just how accurate this integrator method is when applied to small children still remains in doubt. Frontali believes that one should determine the surface area of each individual subject rather than use a formula.

Scammon, Boyd, Klein and Donovan (15) have gone into the question of the surface area of fetuses and children with great care. First Boyd (16) determined the experimental errors inherent in measuring the growing human body and found that the measurement of sitting height used in the Dreyer formula was difficult with children. She says, "Since the determination of standing height has the lowest error, it is the best of the six measurements studied with which to compare other biologic phenomena, such, for example, as vital capacity or surface area.

"Any formula for bodily dimension or function which makes use of the

shorter dimension, body stem, will carry a larger variability than one using standing height, for it will involve multiplying a greater experimental error by a larger constant." Boyd, Scammon and Donovan then made plaster casts of three children and measured their surface areas at leisure. Taking these as a basis and adding the 135 measurements that are found in the literature, they determined the mean relative deviations of various formulas. This series was largely composed of small children, 100 of the measurements being made on subjects under 41 pounds in weight, only 44 on subjects over 41 pounds. The Sage formula showed a mean relative deviation of 7.3% for the whole series but only 5.6% for the groups over 11 kilograms in weight. Using $K = 76.4$ the results were a little better. The best fit was obtained by a formula, $S = W^{0.575} H^{0.275} \times 394.56$, but $W^{0.692} \times 1008$ was almost as good, the mean relative deviation being 5.4%. They conclude: "Surface area in the growing period can be calculated with about equal precision from expressions with constants determined by least squares for weight alone and from expressions devised by the geometric method of Du Bois and based on both height and weight. In this period the relation of surface to weight closely approximates the expression $S = W^{2/3}$."

Klein and Scammon, with apologies, apply the Sage height-weight formula to a series of fetuses varying in weight from 1.26 grams to 2½ kilograms. This is quite a long distance away from the "human pork barrel" who weighed 93 kilograms—still the mean relative deviation was only 21.3%. Deviations of 8.7% and 5.8% were shown by formulas based on weight or on length alone, the best being $S = W^{0.750} \times 5.188$.

Time does not permit us to discuss at length the surface area of animals, but Cowgill and Drabkin (17) who measured the surface of seven dogs noted that a formula with the Sage values for height and length with a constant of 4.381 had an error of only 2.42%. The best fit was obtained by a formula that took into account the nutritive condition of the animal and this could be simplified in the expression $S = W^{0.367} L \times 2.268$. This type of formula when applied to man gave results amost as good as the Sage. Lee, working with rats, obtained his best results with a formula of this same type but got almost as good an agreement with the Meeh formula using a constant of 9.0. Applying this formula to the basal metabolism of the rats studied by Carman and Mitchell (18) he found the basal metabolism only 16–14% lower than that of humans according to square meters of body surface. Benedict and MacLeod (19) found their albino rats averaged about 6% below the level for humans according to surface area. Cowgill and Drabkin, recalculating the surface of two of

Lusk's female dogs, found the average 1.5% below that of women according to the Harris-Benedict standard.

A great deal of work has been done on cattle and swine. Hogan and Skouby (20), obtaining good agreement in one case from the mould and the hide methods, employed them both and worked out for cattle a formula $S = W^{.4} \times L^{.6} \times K$. For cattle L = length from the point of the withers to the end of the ischium and K = 217.02. For swine, the length is taken from the point of the withers to the root of the tail and K = 175. They find their formula more accurate than that of Moulton and much more accurate than the original Meeh. Brody and Elting (12), using the integrator method, measured 96 dairy animals. Good agreement was obtained with the formula $S = 0.15\ W^{.66}$ and they saw no need for putting a linear measurement in the formula. Mitchell (21) has found for Leghorn chickens the formula $S = 8.19\ W^{.705}$ which gives better results than using weight to the $\frac{2}{3}$ power.

In a detailed discussion of all the surface area formulas, Brody, Comfort and Matthews (2) have charted 133 measurements on human subjects made by various investigators. They conclude that for individuals of normal build the equation relating area to height is $A = 240\ H^{.40}\ W^{.53}$ but that good results are also obtained by the formula $A = 1000\ W^{.585}$ leaving height out of the question entirely. They point out that the error in the Sage (Du Bois) formula may be greater than 10%, and print a nomogram based on the formula $A = .024\ H^{.400}\ W^{.538}$ which they believe will give errors less than 10%. This nomogram includes weights of 3 to 100 kilograms. Extending their discussion to animals other than men they believe that in the Meeh type of surface area formula the exponent of weight for different animals should vary from .32 to .72. This indicates that we must accept with caution a great deal of the work that has been done in the past.

The whole question of the significance of the surface area has been discussed by Brody (2) and Mitchell (21). The latter points out the great variability of surface depending upon the position of the animal and the ease with which the skin is stretched. One of his rats with the mould method gave a surface of 432 sq. cm., the unstretched skin, 536 sq. cm., the stretched skin, 630 sq. cm. Mitchell, therefore, states with justice that heat production per sq. meter of body surface possesses no absolute meaning but still may be highly useful for comparative purposes, if measurements are made by the same method. He believes that hide area is just as good as surface area as a unit since it may be considered a maximum surface area. Bohnenkamp (22) goes to the other extreme, insisting that

since the skin has the properties of a black radiator, it is the projection surface that counts and that we should consider only the profile of the body. It would be very interesting if we could contrast in man results calculated according to the actual surface and according to the profile, but this will never be possible since Benedict has shown that surface can be calculated by measuring the profile and multiplying its area by a constant. Brody brings up the point that a great many body components are proportional to the surface. For example, he shows that with dogs varying from 3 to 40 kilograms the weights of the kidneys, liver, lungs, blood, stomach and intestines increase directly with body weight at the same relative rate as does surface area. These facts are pertinent but his arguments drawn from the heat production of the flounder and the metabolism during pregnancy and fasting have little to do with the case. Brody sees no necessity for bringing area into the problem of heat production when one measures not area but weight and height. He does not see how the statement, "Heat production varies directly with body surface" is any better than the statement, "Heat production varies directly with the .56 or with the .67 power of body weight." Mitchell, favoring the other side of the question, in an excellent discussion of the significance of body surface, concludes: "Thus, the characteristic features of warm-bloodedness in animals as they have been observed and reported, render inevitable a close relation between surface area and basal heat output. The relation possesses a physical significance which is entirely ignored in Brody's recommendation that basal metabolism be considered merely as a power function of body weight rather than as a function of body surface." The fact that the weight of the various internal organs, blood volume, cross section of the trachea, etc. vary with the surface area is not at all surprising since one would expect them to depend on the level of the heat production —perhaps the basal heat production, but more probably the average figure for the day. Brody realizes that in the case of man it is impossible to use the weights of these organs as standards of metabolism. Even in the case of experimental animals this proves difficult. Surface area still seems the method of choice as a basis of comparison. In the course of a few years there will be available a large number of accurate surface area measurements, accompanied by basal metabolism determinations on many different species of animals. It is to be hoped that estimations will also be made of the total protoplasmic mass of these animals and we should like to know the relationship of the total nitrogen of the body to the basal heat production. Before we can answer with finality the old question whether or not surface area bears a causal relationship to basal metabo-

lism we need further studies of the responses of different animals to changes in the environmental temperature.

## Metabolism of Children

The subject of metabolism of children is being discussed in a lengthy review by Murlin (23). There is no need of covering the same ground but brief reference should be made to a few of the more important articles that modify our views regarding the so-called "normal standards." It has long been realized that the curve sketched by Du Bois in 1916 and 1917, representing the metabolism of children, is too high, while recent work has indicated that the Benedict and Talbot standards are too low. Thus Sandiford and Harrington (24) found that their general averages between the years of five and seventeen fell between the standards of Benedict and Talbot and the Sage standards of Aub and Du Bois, but noted that the decrease in metabolism was nearly a straight line similar to that found by Benedict and Talbot. The later publication of Boothby and Sandiford (25) confirms the work of Sandiford and Harrington, and their line for boys lies at first halfway between the Talbot and the Sage curves, but meets the Sage curve at the age of fifteen. Blunt, Tilt, McLaughlin and Gunn (26), in a study of 46 girls between the ages of eight and eighteen get results a little higher than those of Benedict. Wang, Kern, Frank and Hays (27), studying 20 normal children in a cot chamber, found the Benedict-Talbot standards all too low by about 10%, in the case of vigorous children. Wang and Kern (28) noted that Benedict standards corresponded to values obtained during sleep. Klugh (29), working in Atlanta on normal children from six to twelve years of age, found his averages were 16% above those of Benedict and Talbot. Rosenblüth (30), studying the age period between eleven and nineteen, found the Benedict-Talbot too low by 15 to 25%. He obtained closer agreement with the Kestner-Knipping normal standards. Ullrich (31) also obtained higher figures for infants.

One of the weakest points in the curve of basal metabolism published by Du Bois in 1914–1915 was a rise in the slope occuring at the ages of twelve to fourteen, just at the onset of puberty. The number of observations upon which this was based was rather small, and the matter remained somewhat doubtful until the fairly numerous confirmations of the last few years. Göttche (32) noted that in about half the children the basal metabolism was raised at puberty. Lax and Petényi (33, 34) also concluded that at the time of puberty the basal metabolism was distinctly raised, obtaining results nearer the Sage curve than that of Bene-

dict and Talbot. Rosenblüth found his largest variations in metabolism occurred between the ages of fourteen and sixteen and ascribed it to the effect of puberty. Kestner (35) believes that the increase in the metabolism before puberty can be ascribed to the relatively short legs and relatively large internal organs. The work of Eckstein and Mommer (36) indicates that the struma of puberty is not accompanied by hyperthyroidism, since their children with struma had about the same basal metabolism as the Kestner-Knipping figures. Topper and Mulier (37, 38) noted in 4 children whose metabolism was studied several times a rise of 10 to 18% coincident with the period of pre-puberty and in 2 other children a fall of 6 to 14% after puberty had been established. They believe that there is a temporary dysfunction of the thyroid at puberty. Topper and Mulier were studying the basal metabolism of over-weight and under-weight children and they employed the von Pirquet standards based on sitting height because they did not wish to use any standard involving weight. 71% of the over-weight but otherwise normal children showed normal basal metabolic rates, the general tendency being somewhat high; their under-weight children had a tendency toward low metabolism, but 70% of them came within a range of ±10%. Wang, Kern, Frank, and Hays (27) found that most of their under-nourished children came within normal limits, but according to height the basal metabolism seemed to decrease with increasing under-weight. Even in 12 under-nourished children who were gaining weight the basal metabolism was apparently normal. (Wang, Kern, and Kaucher) (39).

Kestner and Knipping (40) have adopted the Harris-Benedict multiple prediction formula and have extended it to children. They report remarkable agreement if proper precautions are taken, saying that they seldom find deviations of more than 5% above their calculated figures for healthy persons, or deviations more than 5% below with their normals, except in the case of those who through long training have learned to relax their muscles to an unusual degree. This seems almost too good to be true, but there are confirmatory reports from Schadow (41) and Rosenblüth (30). The latter does not draw the limits quite so close, his range being −5 to +15%, most of his children coming 10 to 15% higher than the Kestner-Knipping standard.

At the present time it would seem advisable to compare the results found in any child with a good many of these normal standards before saying positively that the basal metabolism is beyond the normal range. If we only had more details regarding the physical condition of the children studied in the various reports we could probably narrow down the normal

zone so that practically all of the children would come within about 15% of some good normal standard. Unfortunately, most of the reports fail to give us data of the signs of puberty. The work of Nylin (42) suggests that we should also take into account the rate of increase in height since he has obtained a close correlation between the standard metabolism and the increase in height. With the rapid changes which occur during infancy and childhood one must expect a metabolism much more labile than in the case of adults.

It becomes apparent that as technique improves and as more and more studies become available many factors appear that influence basal metabolism. In most cases the effect is not great, but is enough to defeat an attempt to bring all normal individuals within the zone of $\pm 10\%$ departure from any given standard. Variations of $\pm 15\%$ are being encountered quite frequently and there is a tendency towards even wider minus variations from the standards now generally employed. The greatest care must be used in stigmatizing any one basal metabolism result as beyond the normal range. In the case of groups that are large enough to be statistically significant we must also use care in eliminating the effect of factors which might influence our results in one direction or another. This does not interfere seriously with the practical value of basal metabolism tests, it merely necessitates caution in making sweeping conclusions.

## Mental States

The work of Grafe and his associates on the relationship of mental condition and basal metabolism has been extended during the last few years. Landis (43) has demonstrated the considerable rise due to gastric and rectal discomfort, markedly augmented by the anticipation of painful stimuli. Bauer (44) has pointed out that neuroses not infrequently depress basal metabolism and that the test of the rate is not a quantitative measure of the degree of hyperthyroidism. Henry (45) has departed from the stereotyped method of dividing up the psychoses according to the diagnoses of the disease and has emphasized the importance of the emotional state. Elated, over-active, and over-talkative states are accompanied by an acceleration of the basal metabolism; conversely, depressed, under-active and under-talkative states are accompanied by a retardation. While the apprehensive, tense, and agitated states usually imply an acceleration, this may be counteracted in some cases in which the feeling of depression is intense. Goldwyn (46) noted that hypnosis with suggestion of complete mental and physical relaxation resulted in a lowered metabolism, the average being about 4% below the figures obtained without

hypnosis. One of the most striking studies is that made by Segal, Binswanger, and Strouse (47) on the result of telling patients in the evening that they were going to be operated upon the next morning. With 12 patients who did not suffer from thyroid disease there was practically no change in basal metabolism. The same could be said of a series of 12 patients with hyperthyroidism who had been well treated with Lugol's solution. In the series of patients with hyperthyroidism, before the Lugol's solution had been effective the rise in metabolism in several instances amounted to 20 to 30 points above its previous level. In one patient whose rate before the news of impending operation had been +54%, the figure rose to +182% and then to +270%, with a subsequent fall almost to its previous level. This is a most instructive lesson for clinicians.

### Endocrin Products

Except in the cases of thyroxin and epinephrin, the endocrin products seem to produce no striking changes in metabolism. Missal and Johnston (48) gave whole pituitary by mouth and pituitary extracts intramuscularly over long periods and failed to obtain any significant change in basal metabolism. Schill and von Fernbach (49) obtained similar results.

King (50) reviews the literature on the subject of the artificial menopause and finds no permanent change in the basal metabolism following oöphorectomy. King and Patterson (51) observed the effect of ovarian preparations on the symptoms of the menopause and on the basal metabolism. The rise following administration of corpus luteum was slight and inconclusive, the fresh whole ovary and follicular extract seemed to have no significant effect. Geist and Goldberger (52) were unable to observe any change in the metabolism following castration in women. Gertrud Koehler (53) could not demonstrate any effect of folliculin on basal metabolism.

### Basal Metabolism in Disease

There have been a large number of articles on the subject of metabolism in disease, but very few of them seem to change the views that have been established for several years. Various attempts have been made to classify more definitely that very interesting group of cases with a low basal metabolism apparently not due to hypothyroidism. W. O. and P. K. Thompson (54) studied 66 cases with metabolic rates below −15 after treatment for toxic goiter. They believe that most of the reductions below −25% are myxedema, and that most above this point are not myxedema. Warren (55) has made an analysis of 152 patients showing

low basal values admitted to a sanatorium. Nothing very definite seemed to come from the analysis except that in this group fatigue was most frequently listed as the chief complaint and diagnoses of psychoneuroses, etc. were frequent. Strouse and Binswanger (56) have made a preliminary report of a most interesting group of 32 cases that showed nervous symptoms suggestive of hyperthyroidism without abnormal basal metabolism. Most of these patients showed diffuse thyroid enlargement and tremor. 24 of the 32 were relieved of their symptoms within a few days by the administration of iodin. Strouse suggests that the thyroid may have two functions—one controlling metabolism and the other linked with the nervous system. Mosler and Edelstein (57) have observed a high metabolism in cases of elevated blood pressure and in arteriosclerosis with kidney involvement. Hayasaka (58) did not find increased basal rates in essential or benign hypertension and his patients with secondary contracted kidneys tended to have a reduced rate; those with uremia and malignant hypertension showed some increase. Stander and Peckham (59) noted a slight elevation in pre-eclamptic toxemia and a distinct rise in nephritic toxemia and eclampsia. Strieck (60) has shown that the increased metabolism of leukemia cannot be reduced by pyramidon or quinine. Strieck and Mulholland (61) found the same lack of effect in the high metabolism of carcinoma patients, and the increase in metabolism in carcinoma proved to be proportional to the severity of the clinical picture. Krantz (62) has found in 30 cases of lymphoblastoma an increased metabolism which may be reduced by Roentgen rays and radon. Gessler (63), in a study of 7 cases of endocarditis lenta during afebrile periods of their disease observed an increased metabolism in all, and concluded that this throws a good deal of doubt on the relationship of the van't Hoff law and the increase of metabolism in fever. We must remember, however, that he studied his patients in four-and-a-half-hour periods in a respiration chamber. All of his patients had heart disease though they were not dyspneic; most of them had shown evening fever and several of them were so ill that they died within a few days of the observation. It is very hard for any except well-trained subjects to remain motionless four and a half hours and it is difficult to see how patients who are seriously ill can observe strict basal conditions for this length of time.

## General Reviews

For a discussion of the significance of the respiratory quotient the reader is referred to the monograph by Richardson (64) which embodies the views of the Lusk school. The metabolism of childhood is covered in the

article of Helmreich (65), a splendid series of papers by Levine, Wilson, and their associates (66) and by the forth-coming monograph by Murlin (23). Grafe (67) has made several reviews with references as late as 1927 and Rapport (68) has brought up to date the literature on the interconversion of foodstuffs. The reader who is interested in the effect of the hypophysis should consult the references collected by Raab (69). It is unfortunate that time does not permit of a discussion of the work on animals that has thrown so much light on the whole subject of basal metabolism. One of the most interesting phases of this subject was discussed by Murlin in the first number of this journal.

E. F. D. B.

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